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The Role of Epicuticular Leaf Waxes in Resistance of Plants Against Powdery Mildews

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F I N A L R E P O R T

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Title: "The role of epicuticular leaf waxes in resistance of plants
against powdery mildews"

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3. Abstract.

A 3-years study has shown that epicuticular leaf waxes of plants may possess a major chemical barrier against powdery mildews. Using a newly-developed in vivo bioassay to detect such active compounds we discovered that the immunity of Nicotiana glutinosa to tobacco powdery mildew incited by Erysiphe cichoracearum DC at the >6 leaf stage results from the presence of 2-epiketomanool (designated in previous reports as Compound A) on leaf surface. This compound, when applied to susceptible tobacco plants, caused total inhibition of powdery mildew at a concentration of 2.6 ug.cm⁻² leaf tissue. Resistance to powdery mildew of the tobacco cultivars Kutsaga El, Kutsaga 51E, and TB22 could not be related to external chemical inhibitors on their leaf surfaces.

Immunity of N. tomentosiformis was found to result from the presence of another inhibitory compound, designated as Compound B (yet not identified) which upon application to susceptible tobacco plants inhibited the development of E.cichoracearum. Activity of Compound B was masked by other components in the crude extract of the leaf surface.

Immunity of N. debneyii to powdery mildew resulted from the presence of Compound C on the leaf surface. The structure of Compound C is not known. Another fraction in the epicuticular leaf waxes of this species was inhibitory to Peronospora tabacina (the blue mold agent of tobacco) in vivo but not in vitro.

Resistance of N. tabacum cv. michal to powdery mildew induced by a previous leaf inoculation with P. tabacina could not be traced to a specific inhibitor on leaf surface. However, the major microtopographical changes occurring on P. tabacina-infected leaves suggested that a mechanical barrier may be responsible for this resistance.

Systemic resistance of upper leaves of cucumbers to powdery mildew induced by a previous inoculation of lower leaves by TNV was associated with enhanced lignification of protected-challenged leaves. Changes in epicuticular leaf waxes were not yet determined.

4. Body of report.

- 4.1 Conidial germination of Erysiphe cichoracearum on host and non-host leaf tissue.

See document No. 1 attached.

- 4.2 Cultivar resistance and species immunity of Nicotiana spp. against powdery mildew of tobacco.

See document No.2 attached.

- 4.3 A preformed chemical inhibitor of tobacco powdery mildew on leaves of Nicotiana glutinosa.

See document No.3 attached.

- 4.4 Scanning electron Microscopy of leaf surfaces of tobacco infected with Peronospora hyoscyami f.s.p. tabacina.

See document No.4 attached.

- 4.5 Tobacco necrosis virus induces systemic resistance in cucumbers against Sphaerotheca fulginea.

See document No. 5 attached.

- 4.6 Preformed inhibitors of tobacco powdery mildew on leaves of Nicotiana tomentosiformis and N. debneyii.

N. tomentosiformis (=N.t.) was found to be immune against powdery mildew at all stages of plant growth, whereas some individuals of N. debneyii (=N.d.) were slightly susceptible at the seedling stage.

Crude epicuticular leaf extracts of N.t applied to leaf surfaces of the susceptible tobacco cultivars Michal or Ky 16, before being inoculated with powdery mildew, were not inhibitory to the disease. Nevertheless, when fractionated on silica gel column and used for disease control tests, there was at least one compound (designated compound B, Rf=0.65 in chloroform:methanol 98:2) which was strongly inhibitory (ED99= 5 ug/cm² leaf tissue) to the mildew in vivo. This compound is currently being identified. We hypothesize that crude epicuticular leaf extracts of N.t. contain materials which mask or overcome the inhibitory activity of compound B.

Crude epicuticular leaf extracts of N. debneyii were inhibitory to the powdery mildew fungus upon application to susceptible tobacco leaves. The active ingredient was not yet purified but designated as Compound C.

- 4.7 Preformed inhibitor of Peronospora hyoscyami f.sp. tabacina on leaf surfaces of N. debneyii.

N. debneyii is immune to tobacco blue mold incited by both the Israeli and American pathotypes of P. tabacina. Fungal Conidial germination on leaf surface of N. debneyii was more abundant than on leaf surface of the susceptible tobacco Ky 16.

Crude and fractionated epicuticular leaf extracts of N. debneyii were not inhibitory to conidial germination of P. tabacina in vitro. Using in vivo inoculation tests we discovered that epicuticular leaf waxes of N. debneyii contain a compound (designated as Compound D, Rf=0.83 in n-hexane:acetone 95:5) which was strongly inhibitory to blue mold development on the susceptible tobacco Ky 16. Compound D is currently being identified.

- 4.8 The mechanism of resistance of blue mold infected tobacco to powdery mildew.

Blue mold infected tobacco cv. Michel was found extremely resistant to powdery mildew. Document No.4 shows that such leaves exhibited considerable microtopographical changes. Unfortunately, we could not establish whether these changes may serve as a physical barrier against the powdery mildew fungus. Also we found that crude epicuticular leaf extracts of such leaves were not inhibitory to powdery mildew when applied to Ky 16 or Michal before inoculation with E. chichoracearum. Currently we examine whether fractionated extracts contain any inhibitory substances to powdery mildew.

- 4.9 The effect of 2-epiketomanool derivatives on powdery mildew of tobacco.

The following compounds were obtained from Dr. Enzell (Swedish Tobacco Company, Sweden) and tested for activity against powdery mildew in vivo: 1) Sclareol. 2) manoyl oxide. 3) 8-hydroxymanool 4) dihydroxymanoyloxide. 5) 2-b-hydroxymanoyloxide. 6) epimanoyloxide. Of these compounds only 2-b-hydroxymanoyloxide was inhibitory to powdery mildew of tobacco in vivo.

5. Evaluation of research achievements.

- 5.1 Original objective No.1 (page 3 of the proposal) was only partially achieved. See 4.8.
- 5.2 Objective No.2 was satisfactorily achieved. See 4.1, 4.2, 4.3, and related documents.
- 5.3 Objective No.3 approached achievement; see documents No.2 and 3.
- 5.4 Objectives No.4 was not achieved as such. Some indirect progress in this respect is given in document 4.5.
- 5.5 Objective No.5 gains some achievement from our suggestion to use surface antifungal compounds such as 2-epikelomanool as a trait to be incorporated into susceptible tobacco cultivars in breeding tobacco against powdery mildew.

6. Description of cooperation.

During his visit to Israel thorough discussions were conducted with Dr. J. Kue. Similar discussions were conducted upon Dr. Cohen's visit to the U.S.

Work under Section No.4.7 was done in full cooperation in both Israel and the U.S. Work under section No. 4.5 was inspired by Dr. J. Kue Studies.

9.8 Publications.

- 8.1 Bashen, B. and Cohen, Y. 1983. Tobacco necrosis virus induces systemic resistance in cucumbers against Spaerotheca fuliginea. Physiol. Plant Pathol. 22 (in press).
- 8.2 Cohen, Y. 1982. Cultivar resistance and species immunity of Nicotiana spp. against powdery mildew of tobacco. in: Dunez, J. (Ed.) La Selection des Plantes pour la Resistance aus Maladies. Colloque Frances-Israelien. Bordeaux Mars 1982, pp. 143-156.
- ✓ 8.3 Cohen, Y., Eyal, H., Sklarz, B. and Goldschmidt, Z. 1983. A preformed chemical inhibitor of tobacco powdery mildew. Physiol. Plant Pathol. 22 (in press).
- 8.4 Skubatz, H. and Cohen, Y. 1981. Conidial germination of Erysiphe cichoracearum on host and non-host leaf tissue. Phytoparasitica 9: 241-242 (Abstr.)

✓9.5 Skubatz, H. and Cohen, Y. 1983.

Scanning electron microscopy of leaf surfaces of tobacco infected with
Peronospora hyoscyami f.sp. tabacina.

Canadian J. Plant Pathol. (in press).

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Tobacco necrosis virus induces systemic resistance
in cucumbers against Sphaerotheca fuliginea

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ABSTRACT

Cucumber (Cucumis sativum) plants locally infected with tobacco necrosis virus (TNV) in cotyledons or lower true leaves were partially protected against powdery mildew, incited by Sphaerotheca fuliginea, in upper leaves. In TNV-infected plants colony development and conidial production of S. fuliginea were reduced by about 65 and 85%, respectively, compared to TNV-free plants. Protection in leaf 2 was evident at 48-96 hr after inoculating leaf 1 with TNV. Of the 5 cultivars tested, Poinsett, which was partially resistant to powdery mildew, expressed the highest degree of induced resistance. TNV did not induce resistance to powdery mildew in cantaloupe (Cucumis melo) nor in squash (Cucurbita moschata) plants. In two separate field trials, cucumber plants were partially protected against challenge inoculation with S. fuliginea by a prior inoculation with TNV. Histochemical observations indicated that lignification was more frequent in challenged-protected than in challenged-unprotected plants. Localized infection with TNV failed to protect cucumbers against downy mildew incited by Pseudoperonospora cubensis.

Introduction

Tobacco necrosis virus is reported to induce systemic resistance in cucurbits against Colletotrichum lagenarium, Pseudomonas lachrymans, tobacco necrosis virus [8,9] and Cladosporium cucumericum [12]. However, no quantitative studies have been reported on the effect of a prior inoculation with TNV on the subsequent development of obligate fungal pathogens or of pathogens penetrating through stomatal openings. Hwang and Heitefuss [7] recently reported that infection of lower leaves of barley with Erysiphe graminis f. sp. hordei protected an upper leaf from disease caused by a subsequent inoculation with this pathogen.

This paper provides evidence that a localized infection with TNV can systemically protect cucumber plants against powdery mildew incited by Sphaerotheca fuliginea but not against downy mildew incited by Pseudoperonospora cubensis.

Materials and Methods

Plants, pathogens and inoculations

Most experiments were conducted with the cucumber (Cucumis sativum) c.v. Bet-Alfa. The cucumber cultivars Poinsett, SMR-58, Marketer, and Straight-8, the cantaloupe (Cucumis melo) cultivar Haogen, and the squash (Cucurbita moschata) cultivar Sihi-Lavan were used in some experiments.

Plants were grown in 15 cm plastic pots in the greenhouse (20-32°C) and were inoculated at the 2 true leaf stage, unless stated otherwise.

Tobacco necrosis virus (TNV) was obtained from Dr. Sarah Spiegel (Volcani Institute for Agricultural Research) and maintained on cucumber plants in a growth chamber at 20°C.

The virus inoculum was prepared by grinding with a pestle and mortar infected cucumber cotyledons in tap water (1 ml/cotyledon). The homogenate was rubbed onto cotyledons and/or leaf 1 which have been dusted with carborundum. Control plants were dusted with carborundum and inoculated with the homogenate from healthy cotyledons. Inoculated plants were washed with water about 5 min after inoculation, and transferred to a 25°C cabinet (50-60% r.h.) illuminated 12 h/day with fluorescent light (supplemented with incandescent bulbs) at an intensity of about 150 $\mu\text{E.m}^{-2}.\text{s}^{-1}$. TNV lesions usually appeared 4 to 5 days after inoculation.

Sphaerotheca fuliginea (Schlecht ex. Fr.) Poll. and Pseudoperonospora cubensis (Berk. & Curt.) Rost., the two obligate fungal pathogens used for challenge inoculations, were collected from the field in 1980 and 1978, respectively, and maintained on cucumber plants in growth cabinets at 20°C.

To challenge inoculate with powdery mildew, infected plants bearing young colonies were shaken over the test plants. Inoculated plants were then transferred to 25°C cabinet (see above) for disease development. Records of parasite development on upper leaf surfaces were taken at intervals between 7 and 27 days after inoculation using one or more of the following techniques: (a) counting the number of pustules per leaf (employed when discrete colonies were distinguishable); (b) visual assessment (0-5 scale) of the total area of mildew coverage on the aerial parts with 0 = no mildew observed and 5 = full coverage of leaf surface with mildew (employed when colonies had coalesced); and (c) counting the number of conidia produced per unit leaf area. Leaf discs

(2 cm in diameter) were placed in a known volume of formalin:glacial acetic acid:ethanol (5:5:90, v/v) and conidia were counted using a haemocytometer. In some experiments fungal development was examined microscopically in leaf discs clarified in ethanol and stained with 0.1% trypan blue in lactophenol.

Challenge inoculation with downy mildew was done by placing 5 μ l of a sporangial suspension (50,000 sporangia/ml) on the lower leaf surfaces of the test plants. Plants were incubated in a moist atmosphere in the dark at 15°C for 4, 8, or 20 hr, and then transferred to 20°C cabinet for lesion development [2]. The number and size of lesions which developed were recorded on the 7th day after inoculation. Sporulation of P. cubensis on challenged plants was quantitated as described before [2].

Histochemical observations

Protected and unprotected cucumber plants (cultivars Bet-Alfa and Poinsett) challenged with S. fuliginea were used for the histochemical observations. Leaf discs (1 cm in diameter) were taken daily from inoculated plants during the first week after inoculation and placed in boiling 95% ethanol until clarified (usually 30 min). Discs were then treated with 1% phloroglucinol in 70% ethanol for 6-7 min, mounted on glass slides, treated with 32% HCl for 2-3 min and examined microscopically. Lignified cell walls appeared red [5].

Statistical analysis

Due to the technique employed to challenge inoculate plants with powdery mildew (dusting spores on the leaves), disease development was very variable. In most experiments at least 15 plants/treatment were used. Standard deviation of the mean was calculated for each treatment

and t-tests were run between treatments for significant difference determination.

Results

Protection of leaf 1 and leaf 2 of cv. Bet-Alfa

Cotyledons were inoculated with TNV at two different concentrations giving means of 6.5 and 18 lesions per leaf. One week later leaf 1 was challenged with either S. fuliginea or P. cubensis. Fig. 1A shows the development of powdery mildew on these plants one week after challenge. The number of colonies was reduced by 34 and 62% in plants bearing 6.5 and 18 TNV lesions, respectively, compared to virus-free plants. Conidial yields were reduced by 75% and 80%. When leaf 1 was inoculated with TNV and leaf 2 was challenged one week later, similar results were obtained (Fig. 1B). In both experiments fungal sporulation was suppressed to a greater extent than pustule production.

Neither experiments showed protection against P. cubensis with the number of lesions, their size and sporulation being the same on TNV-inoculated and control plants.

Protection of leaves 4-6 of cv. Bet-Alfa

Leaf 3 on 4-leaf plants was inoculated with an inoculum of TNV giving about 50 lesions/ leaf and one week later plants having developed a further two leaves, were challenged with S. fuliginea. Fungal development was recorded 7 and 14 days after inoculation.. Pustule production 7 days after inoculation on leaves 4,5 and 6 in TNV-infected plants was reduced, relative to virus-free plants, by 69,54 and 30%, respectively (Fig. 2A), while corresponding conidial yields were reduced by 85, 93 and 80% (Fig. 2B). Most of these differences between protected and

unprotected plants were highly significant ($P < 0.01$). As at 14 days after inoculation, pustules coalesced and their counting was not feasible, only conidial yields were determined (Fig. 2C). These were reduced by 53,55 and 48% in leaves 4,5 and 6, respectively, of protected plants compared to the corresponding leaves of unprotected plants. Protected plants also exhibited resistance to mildew on hypocotyls, stems and petioles to an extent of 53,50 and 34%, respectively, based on the relative coverage of these organs with mildew.

Protection of various cultivars

Cucumber plants of the cultivars Marketer, SMR-58, Bet-Alfa, Straight 8 and Poinsett were inoculated with TNV on leaf 1 (about 30 lesions/leaf) and a week later challenged in leaf 2 with either S. fuliginea or P. cubensis. At 7 days after inoculation no differences in downy mildew development were recorded between TNV-infected and TNV-free plants. At 11 days, conidial yields of S. fuliginea were reduced in TNV-infected plants, compared to TNV-free plants, by about 50-90%, depending on the cultivar, with Poinsett exhibiting the highest degree of protection (Fig. 3). Microscopical examinations showed that in Poinsett the number of conidiophores of S. fuliginea in protected plants was reduced by about 92% compared to unprotected plants.

Interval between protection and challenge

Two-leaf cucumber plants (cv. Bet-Alfa) were inoculated with either TNV or healthy leaf extract in leaf 1. At various intervals after inoculation with the virus leaf 1 was excised. Plants were challenged with S. fuliginea in leaf 2 at 7 days after viral inoculation, and assessed for powdery mildew development after additional 7 days. When colonies on leaf 2 were counted, protection was evident 96 hr after inoculating leaf 1 (leaf 2 of unprotected and protected plants had an average of 145 ± 100 and 47 ± 19 colonies, respectively, $P < 0.025$), but when conidial yield per unit leaf area was measured, protection was evident 48 hr after inoculating leaf 1 (52.9 ± 19.6 and $30.4 \pm 14.9 \times 10^3$ conidia/cm² in leaf 2 of unprotected and protected plants, respectively, $P < 0.05$). Protection gradually increased as the interval period between inoculating leaf 1 and excision leaf 1 expanded (Fig. 4).

Field trials with cv. Bet-Alfa

In trial 1 (July 1981), 30 two-leaf cucumber plants were inoculated with TNV in leaf 1 and 30 plants remained uninoculated. At 7 days after inoculating leaf 1, all plants were transplanted to the field, in which a natural source of powdery mildew existed. Disease was recorded at 10, 17 and 24 days after transplanting. At 10 days, plants had 4-5 leaves and the average number of pustules/plant in unprotected and protected plants was 184 ± 92 and 63 ± 42 (66% protection, $P < 0.05$), respectively. At 17 days, plants reached the 9-10 leaf stage and the average degree of protection was 53% (Fig. 5). No protection was observed at 24 days after transplanting, when plants had 13-14 leaves.

In trial 2 (August 1981), 35 plants at the 2-leaf stage were inoculated with TNV on leaf 1 and 35 remained uninoculated. At 4 days after inoculating leaf 1, all plants were transplanted in the field. Disease records were taken at 11, 18 and 27 days after transplanting. Number of powdery mildew pustules/plant on unprotected and protected plants were 15 ± 12 and 3 ± 5 , respectively, at 11 days ($P < 0.01$), and 136 ± 85 and 81 ± 48 , respectively, at 18 days ($P < 0.01$). By the 27th day after transplanting, no differences in disease development were recorded between protected and unprotected plants.

Microscopical examinations

Leaf 2 of protected and unprotected plants (cv. Bet-Alfa) was challenged with S. fuliginea at 7 days after inoculating leaf 1. Discs from the challenged leaves were excised at various intervals after challenge and either clarified in ethanol and stained with 0.1% trypan blue in lactophenol, or stained with phloroglucinol-HCl for lignin determination. Conidial germination with 1 and 2 germ tubes at 18 and 30 hr after inoculation, respectively, proceeded equally well in both protected and unprotected plants. The development of germ tube 3, at 48 hr, was inhibited in 69% ($\pm 16\%$, in 4 separate experiments) of the germinating conidial population in the protected plants relative to the unprotected plants. Hyphal growth at 72 and 96 hr was poorer in protected plants than in control unprotected plants. Lignification in epidermal cells was more frequent in protected than in unprotected plants up to 72 hr after inoculation with S. fuliginea. At 96 hr lignification was abundant in both protected and unprotected plants (Table 1).

Discussion

Systemic resistance in plants was demonstrated to be induced against many organisms [1,4,9,10]. There is no evidence in the literature that such resistance can be induced in cucumbers against obligate fungal pathogens. Hwang and Heitefuss [7] showed that in barley infection of leaf 1 and 2 with Erysiphe graminis f. sp. hordei induced resistance in leaf 3 against the same pathogen. An incompatible race of the fungus, which produced a hypersensitive reaction, was more effective than a compatible race, and a barley cultivar with adult plant resistance was better protected than a susceptible cultivar. Beretta et al. [1] showed that coffee plants could be protected against rust at a distance from the site of applying water used to wash autoclaved urediospores of Helmileia vastatrix.

This paper demonstrates that TNV induced systemic resistance in cucumbers against powdery mildew incited by S. fuliginea under both laboratory and field conditions. Resistance was more profound in a partially resistant cultivar than in susceptible cultivars, thus confirming other reports on amplification of resistance in genetically resistant cultivars [8, 10]. Sporulation of the challenge fungus, in plants previously inoculated with TNV, was inhibited to a larger extent than colony formation (Figs. 1,2,4), and in some experiments were inhibited by 85 and 75%, respectively (Fig. 4). Similar to other systems [4,10] protection (colonywise) was evident at 4 days after inoculating leaf 1 with the virus, although a significant reduction in conidial yield of the challenge fungus was determined as early as 48 hr after virus inoculation.

No protection whatsoever, could be induced by TNV in cucumbers against P. cubensis, the causal agent of downy mildew. Other inducing organisms we used (Colletotrichum lagenarium, Cladosporium cucumerinum) failed also to elicit such protection (data not presented). S. fuliginea and P. cubensis differ in their mode of penetration: while the former fungus penetrates directly through the periclinal epidermal cell walls, the later penetrates exclusively through stomatal openings [3]. This brought us to believe that the epidermis has a role in induced resistance of cucumbers, a suggestion raised earlier by Richmond et al. [11] and Jenns [8]. Our preliminary observations (Table 1), and those reported by Hammerschmidt and Kuć [6], showing a higher rate of lignification of epidermal cells in protected compared to unprotected plants, give support to this idea. More microscopical observations have to be done to establish whether penetration of S. fuliginea is inhibited in protected compared to unprotected plants. Except for the pathosystem coffee - H. vastatrix [1], we know of no other case in which systemic resistance was induced against stomatal penetrating fungi.

Our results showed that TNV did not induce resistance against powdery mildew in both cantaloupe and squash. In cantaloupe the reason may be explained by the failure of the virus to produce local lesions, in squash the reasons remain obscure.

Acknowledgements. This research was supported by a grant from the United States-Israel (Binational) Agricultural Research and Development Fund (BARD) granted to Y. Cohen and J. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, Ky.

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Table 1. The effect of infecting the first true leaf of two cucumber cultivars with TNV on the lignification of epidermal cells in leaf 2 challenged with Sphaerotheca fuliginea

Mean number ($\pm$ S.D.) of epidermal cells per disc showing lignification at indicated hours after challenge inoculation

Treatment on leaf 1 ^a	cv. Bet Alfa			cv. Poinsett		
	24	48	72	24	48	72
Unprotected	0	0.5 $\pm$ 1.3	3.6 $\pm$ 4.4	0.1 $\pm$ 0.3	1.2 $\pm$ 3.9	1.5 $\pm$ 2.4
Protected	0	1.3 $\pm$ 7.2	12.5 $\pm$ 10.5	0.3 $\pm$ 0.7	9.7 $\pm$ 15.9	16.7 $\pm$ 27.4

^aLeaf 1 of 2-leaf plants was inoculated with TNV. Control plants were inoculated with virus-free leaf homogenate. Seven days after inoculation, leaf 2 was inoculated with S. fuliginea. Eight leaf discs (1 cm diam.) were removed from leaf 2 of 4 plants and the lignified epidermal cells counted after staining with phloroglucinol-HCl.

Legend to Figures

- Fig. 1. Development of Sphaerotheca fuliginea on (A) leaf 1 or (B) leaf 2 of cucumber plants inoculated 2 weeks earlier with TNV of various concentrations on cotyledons (A) or leaf 1 (B). Values are means from 15 plants/treatment. Asterisks indicate that means differ significantly from virus-free plants, *P < 0.05, ** P < 0.025 , and P < 0.01.
- Fig. 2. Development of Sphaerotheca fuliginea colonies on cucumber plants at the 6-leaf stage inoculated with TNV on leaf 3 two or three weeks earlier. Numbers on bars represent standard deviation of the mean (n=28). Asterisks indicate significant differences between virus-free plants (open bars) and TNV-infected plants (dashed bars).
- Fig. 3. Development of Sphaerotheca fuliginea on leaf 2 of five cucumber cultivars inoculated with TNV on leaf 1. Plants were challenge inoculated with S. fuliginea one week after being inoculated with TNV. Fungal sporulation was recorded one week after challenge. Numbers on bars represent standard deviation of the mean (n=15). Asterisks indicate level of significance in differences between virus-free (open bars) and virus-infected (dashed bars) plants.
- Fig. 4. The effect of excising leaf 1 inoculated with TNV, at various intervals after inoculation, on the development of Sphaerotheca fuliginea in leaf 2. Values represent mean (n=8) inhibition of the fungus on TNV-inoculated plants relative to virus-free plants.
- n.s = non-significant difference. Asterisks indicate level of significance in differences between virus-inoculated and virus-free plants.

Fig. 5. Powdery mildew development on protected (TNV-infected in leaf 1) and unprotected (TNV-free=CK) cucumber plants (cv. Bet Alfa) under field conditions. Two-leaf plants were inoculated with TNV in leaf 1. One week later, plants were transplanted in the field together with plants not inoculated with TNV. A natural source of powdery mildew existed in the field. Fungal development was assessed 17 days after transplanting. Two and three asterisks represent significant differences at 0.025 and 0.01 level, respectively, between corresponding leaves of protected and unprotected plants (n=30).

Evaluation of Research Achievements

This research has shown that species immunity in Nicotiana spp. and cultivar resistance in Nicotiana tabacum to powdery mildew incited by Erysiphe cichoracearum results from different mechanisms. Whereas species immunity was associated with the presence of external prohibitions on leaf surface, that of cultivar resistance was associated with hypersensitive response (HR). It was shown that immunity of N. glutinosa is attributed to the trichome diterpene epiketomanool ($C_{20}H_{32}O_2$) which superimposes at seedling stage another unidentified mechanism for resistance. Immunity of N. tomentosiformis and N. debneyi was associated with the presence of other, different, prohibitions yet unidentified. Resistance of tobacco cultivars was not associated with the presence of external prohibitions but mostly from HR. We hypothesized that surface prohibitions interfere with cutinase activity (and possibly other penetration enzymes) secreted during conidial germination~~s~~.

Our study has shown that tobacco plants susceptible to powdery mildew could be protected from the disease by a single spray of epiketomanool extracted from N. glutinosa.

Resistance induced in tobacco against powdery mildew by a previous inoculation with downy mildew (incited by Peronospora tabacina) was shown to be associated with the formation of a heavy waxy coating on leaf surface of the induced plants.

The overall conclusion of the research is that epicuticular leaf substances may be of crucial importance in defence against powdery mildews. Some of these compounds may be of practical use in the future.