Oecologia (1998) 115:523–530

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Plant-mediated interactions between insects and a fungal plant pathogen and the role of plant chemical responses to infection

Received: 28 September 1997 / Accepted: 9 February 1998

Abstract Diverse organisms simultaneously exploit plants in nature, but most studies do not examine multiple types of exploiters like phytophagous insects and fungal, bacterial, and viral plant pathogens. This study examined patterns of induction of antipathogenic peroxidase enzymes and phenolics after infection by the cucurbit scab fungus, *Cladosporium cucumerinum*, and then determined if induction mediated ecological effects on *Colletotrichum orbiculare*, another fungal pathogen, and two insect herbivores, spotted cucumber beetles, and melon aphids. Peroxidase induction occurred in inoculated, 'local,' symptom-bearing leaves 3 days after inoculation, and in 'systemic,' symptom-free leaves on the same plants 1 day later. Phenolics were elevated in systemic but not in local leaves 3 days after inoculation. Detached systemic leaves from plants inoculated with *C. cucumerinum* developed significantly fewer and smaller lesions after challenge with *C. orbiculare*. Spotted cucumber beetles did not show consistently significant preferences for infected versus control leaf disks in comparisons using local or systemic leaves, but trends differed significantly between leaf positions. In no-choice tests, beetles removed more leaf area from local but not from systemic infected leaves compared to control leaves, and melon aphid reproduction was enhanced on local infected leaves. In the field, cucumber beetle and melon aphid densities did not differ between infected and control plants. Antipathogenic plant chemical responses did not predict reduced herbivory by insects. Other changes in metabolism may explain the positive direction and spatially dependent nature of plant-mediated interactions between pathogens and insects in this system.

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Key words Cucumber · Pathogen induction · Peroxidase · Phenolics · Herbivory

Introduction

Plants experience parasitism and predation by a diverse suite of organisms ranging from viruses to large mammals, and these exploiters rarely exist in isolation from one another. A key issue for plant survival is the degree to which different exploiters of plants influence each other (Hammond and Hardy 1988; Benedict and Chang 1991; Edwards et al. 1991; Hammerschmidt 1993). The simultaneous or sequential presence of multiple herbivores on a plant may exert selection pressure favoring certain modes of plant-induced responses and may govern the dynamics of exploiter populations (Williams and Myers 1984; Faeth 1986; Hunter 1987; Mopper and Simberloff 1995). Fungal, bacterial, and viral pathogens commonly rely on chemically dynamic plants for their own survival, and often share their hosts with insect herbivores. Few studies have coupled chemical changes induced by pathogen infection with the ecological effects of infection on insects (Karban et al. 1987; Krischik 1991) and few have examined effects in both laboratory and field environments. Crop plants provide good models for such studies because numerous pathogens and insects individually impact plant growth and reproduction and co-occur at relatively high densities. Plant-mediated 'cross-effects' between pathogens and insects may occur often in these systems and may have ecological consequences applicable to natural ecosystems.

A major part of the chemical response of cucumber, tobacco, soybean, and other plants to pathogens consists of numerous classes of pathogenesis-related (PR) enzymes, inlcuding peroxidases (Smith and Hammerschmidt 1988; Ye et al. 1990; Stermer 1995). Plant-wide induction of peroxidases progresses up or down the shoot from an inoculated 'local' leaf to distant 'systemic' leaves on the same plant (Hammerschmidt et al. 1982;

Hammerschmidt and Yang-Cashman 1995), possibly reducing pathogen spread from an initial infection point (Hammerschmidt et al. 1984). Elevated peroxidase activity is correlated with systemic acquired resistance (SAR), which refers to the reduced severity of lesions or other symptoms of a 'challenge' pathogen applied to plants previously inoculated elsewhere with the same or a different pathogen (Ross 1961; Hammerschmidt and Yang-Cashman 1995). Peroxidases increase after insect or artificial wounding in cucumber (Miller and Kelley 1989; Svalheim and Robertsen 1990) and corn (Dowd and Norton 1995), but their functions as defenses against insects are uncertain. These enzymes might mediate interactions arising from prior microbial infection by decreasing host plant quality for insects, due to the toxicity of oxidized metabolites and free radicals and/or increases in leaf toughness (Duffey and Felton 1991).

In many plants, phenolics constitute another chemical response to pathogen infection. Phenolics are often toxic to fungal pathogens in vitro and accumulate locally around infection sites (Nicholson and Hammerschmidt 1992; Cherif et al. 1994), leading to varying levels of lignin deposition, necrosis, and resistance (Hammerschmidt and Kuc 1982; Lagrimini 1991). Many types of phenolics are induced by insects and alter insect feeding, survival, or reproduction (Appel 1993; Duffey and Stout 1996). Phenolic induction after pathogen infection may influence insect exploitation through changes in leaf toughness (Hammond and Hardy 1988), toxicity of phenolic metabolites (Benedict and Chang 1991; Kogan and Fischer 1991) or nutritional values of hosts (Mattson 1980; Goodman et al. 1986).

Given that chemical changes like peroxidase and phenolic induction occur after infection, plant pathogens might be expected to defend plants against insects. In fact, infection leading to cell necrosis, yellowing due to chloroplast damage, or wilting may result in increases, decreases, or no changes in insect feeding and reproduction (Table 1). With the exception of soybean phenolic phytoalexins (Kogan and Fischer 1991), none of the studies in Table 1 examined antipathogenic chemical responses to infection like peroxidases and phenolics, and only some determined whether infection led to SAR to other pathogens before studying effects on insects. Insects were usually allowed to feed on systemic leaves without symptoms or on whole plants, and usually at only one time point after infection. The existence of cross-effects of infection on insects may depend on leaf location because symptoms like necrotic lesions could alter host suitability as they develop on inoculated leaves, if changes in defenses and nutrients accompany development (Goodman et al. 1986; Barbosa 1991; Hatcher 1995).

Table 1 shows that cucumber (*Cucumis sativus* L.) has been a popular study system, probably because of the large number of pathogens known to induce chemical changes and SAR to other pathogens. In this study, I examined whether plant chemical changes could mediate interactions between herbivores and pathogens of one genotype of cucumber. I assessed the effects of infection on feeding and reproduction of two common insect exploiters of cucumber using local, symptomatic and systemic, non-symptomatic leaves. I extended information gained in controlled bioassays to the field, to see if the presence of an assemblage of pest insects and pathogens in their natural abiotic environment altered the nature of interactions uncovered in greenhouse-grown plants.

Materials and methods

Plants, pathogens, and insects

Cucumber (*C. sativus* L., cv. Straight 8) seeds were sown in 2-Liter pots in Metromix 250 soil (Scotts-Sierra, Marysville, Ohio), watered regularly, and fertilized 2 weeks after planting with Peters N:P:K 20:20:20 fertilizer plus micronutrients (Grace-Sierra, Malpitas, Calif.). Plants were maintained under natural light in a

Table 1 Summary of studies of cross-effects of infection in plants on insect herbivores (*F* fungus; *V* virus; *Y* effect observed, followed by direction (+/−); *N* none; *nd* not determined)

Reference	Plant	Pathogen used	Systemic acquired resistance	Feeding	Effect on reproduction
Apriyanto and Potter (1990)	Cucumber	V	Y	N (whitefly) N (caterpillar) Y+ (beetle)	Y− (mite)[a]
Ajlan and Potter (1991)	Cucumber	F	Y	nd	N (mite) N (aphid)
Blua and Perring (1992)	Zucchini	V	nd	Y− (aphid)	Y− (aphid)
McIntyre et al. (1981) Ajlan and Potter (1992)	Tobacco	V	nd	nd	Y− (aphid) N (aphid)
Hart et al. (1983) Kogan and Fischer (1991)	Soybean	F	Y	Y− (beetle) N (caterpillar)	nd
Karban et al. (1987)	Cotton	F	nd	nd	Y− (mite)
Hatcher et al. (1994)	*Rumex*	F	nd	Y+ (beetle)	Y− (beetle)
Lewis (1979)	Sunflower	F	nd	Y+ (grasshopper)	nd

[a] Effect restricted to symptom-bearing half of inoculated leaves

greenhouse (25–30°C). For the field study, seedlings were grown until they had one true leaf in the greenhouse and then transplanted into rows of black plastic mulch in a 30×60 m field plot at the Russell Larson Agricultural Research Station at Penn State University. Plants were watered via drip irrigation as needed throughout the field season.

Cladosporium cucumerinum, which causes cucurbit foliar necrotic scab, and *Colletotrichum orbiculare*, which causes cucurbit foliar necrotic anthracnose, were cultured as in Staub and Kuc (1980) and Hammerschmidt et al. (1976), respectively, and used as 7-day-old cultures to prepare spore suspensions, adjusted to 3×10^6 spores/ml (*C. cucumerinum*) or 9×10^5 spores/ml (*C. orbiculare*). Both of these doses are within ranges used in other studies of induced resistance and SAR to pathogens (Staub and Kuc 1980), but may be higher than doses encountered by plants in the field via water splashing, the chief mode of transport for spores of both fungi (MacNab et al. 1983).

Diabrotica undecimpunctata howardi Barber (Coleoptera: Chrysomelidae), spotted cucumber beetles, were reared on corn roots as larvae (French Agricultural Inc., Lamberton, Minn.). Newly emerged adult beetles were allowed to feed on sweet potato and acorn squash for 1–3 days prior to the start of bioassays. Melon aphids (*Aphis gossypii* Glover) (Homoptera:Aphididae) were maintained on one to two leaf cucumber seedlings changed every 2–3 weeks.

Inoculations

Three- to four-leaf-stage (17–19 days old) cucumber plants from the greenhouse were randomly assigned to treatments. Treatment plants were sprayed on the growing tip and the topmost expanding leaf (local leaf) with approximately 250 µl of spore suspension. The sytemic leaf (lowest leaf on the shoot) was immune to infection by *C. cucumerinum* (Staub and Kuc 1980). Control plants received sterile deionized water. All plants were kept at 100% relative humidity at 20°C for 2 days, then returned to the greenhouse. The topmost expanding leaf developed necrotic lesion symptoms by the 3rd day after inoculation in treatment plants, and symptoms on this leaf became more widespread over time. Systemic leaves on treatment plants did not develop symptoms within 6 days of inoculation.

Chemical extraction and analysis

Local and systemic cucumber leaves from inoculated plants, and leaves of the same ages from control plants, were harvested (3, 4, 5, and 6 days after inoculation), frozen in liquid nitrogen, and stored at −20°C (four to five plants per treatment per day). For peroxidase analysis, leaves were homogenized in 5 ml/g fresh weight 0.01 M phosphate buffer (pH 7.0), centrifuged, and the supernatant used for spectrophotometric assays employing a Beckman DU 7400 (Beckman, Fullerton, Calif.). Total protein was assessed with bovine serum albumin as the standard (Bio-Rad, Hercules, Calif.). Peroxidase activity was measured as in Hammerschmidt et al. (1982) for 1 min at 470 nm with guaiacol substrate. Since total protein did not differ between treatments or leaf locations, peroxidase data were expressed as the change in activity per mg protein^{-1} min^{-1}.

Extractions of cucumber leaves for phenolics were completed as in Rossiter et al. (1988) with modifications (Cherif et al. 1994). Lyophilized leaves were ground in a Cyclone Mill (UDY, Fort Collins, Colo.) and extracted with diethyl ether to remove pigments. The powder was briefly dried under nitrogen and extracted with 50% methanol containing 0.001 M L-ascorbic acid. The extract was centrifuged and the supernatant dried to half volume under reduced pressure to remove methanol. The reducing potential of extracts, indicative of phenolic content, was determined with the Folin-Denis procedure (Waterman and Mole 1994). Data for samples were compared to a standard curve made using partially purified phenolics from Straight 8 cucumber. Purification involved extracting lyophilized leaf powder as above, mixing the extract with a Sephadex slurry in methanol and extracting with 95% ethanol and 50% methanol. The methanol fraction was reduced in volume, the aqueous extract lyophilized and the dry residue collected and dissolved in water.

SAR bioassays

In three trials, the systemic leaf (lowest leaf on the shoot) on cucumber plants was detached 6 days after *C. cucumerinum* infection and inoculated with five 50-µl drops of *C. orbiculare* conidial spore suspension. Leaves were misted with water and placed in 20-cm clear-plastic boxes. Lesion diameters were measured 7 days later with calipers, and an estimate of total necrotic leaf area in cm^2 was taken with a grid laid over the leaf (0.36-cm^2 squares).

Beetle dual-choice bioassays

Infected and control plants were randomly paired, and local and systemic leaves and controls were harvested 3–6 days after *C. cucumerinum* infection (three to four plant pairs per day). Leaf disks were cut with a no. 11 cork borer (area approx. 170 mm^2) and arranged randomly in 9-cm petri dish arenas (two disks per treatment per arena, four arenas per plant pair for each leaf location) containing moistened filter paper and four spotted cucumber beetles, which were then allowed to feed for 10 h (the time needed to consume approx. 50% of all disk area available). Disk area consumed was estimated with the Optomax V system (Analytical Measuring Systems, UK) attached to an Ikegami ITC-510 camera (Ikegami, Tokyo, Japan).

Beetle and aphid no-choice assays

Local and systemic leaves and corresponding controls were harvested and placed in water-filled tubes 5 days after *C. cucumerinum* infection, in two experiments. The first trial used separate sets of plants and insects for local and systemic leaves, while the second experiment involved local and systemic leaves harvested from the same plants, allowing for comparisons between the two leaf positions as well as between infected and control treatments. Twenty adult spotted cucumber beetles were caged with one leaf in 20-cm clear-plastic boxes (five boxes per treatment per leaf position) and were supplied fresh leaves of the same position from other inoculated plants each day for 5 days. Leaf area before and after 24 h exposure was measured with a Li-Cor leaf area meter (Li-Cor, Lincoln, Neb.) and summed across days. In separate boxes, one apterous newly emerged adult female melon aphid was placed on a local- or systemic-infected or corresponding control leaf and transferred to a fresh leaf daily for 5 days. Number of offspring were counted each day and summed.

Field study

Cucumber seedlings (10–11 plants per treatment) were randomly assigned to treatments in two separate common garden plots and were inoculated as in greenhouse experiments. Plants were allowed to grow until they had one six- to eight-leaf vine (2 weeks) before beginning weekly observations, which were made in the early morning (0700–0900 hours), when cucumber beetles were most active (personal observation). Numbers of cucumber beetles (spotted cucumber beetles and striped cucumber beetles, *Acalymma vittata*), and melon aphids were counted and the counts divided by the number of leaves examined on the plant to obtain densities of insects per leaf. By the 6th week after inoculation, many plants were showing symptoms or mortality characteristic of bacterial wilt

or fungal powdery mildew diseases, ending the study. Preliminary analyses did not indicate an impact of early season inoculation on the occurrence of these later diseases (for wilt, categorical repeated measures $\chi^2 = 1.84$, $P = 0.76$, $df = 4$; for powdery mildew $\chi^2 = 3.23$, $P = 0.52$, $df = 4$).

Statistics

Normality of data was verified with Wilk's lambda ($P > 0.05$) (SAS 1990). Three-way univariate ANOVAs examined the effects of *C. cucumerinum* infection, leaf position, day of harvest, and interactions on peroxidase activity and phenolic levels, using PROC GLM (SAS 1990) and type III sums of squares. Since these analyses revealed significant interaction terms, separate one-way ANOVAs were run for each day and leaf location with fungal treatment as the main effect. Data from SAR tests using *C. orbiculare* and no-choice beetle and aphid bioassays were examined with one-way ANOVAs. Dual-choice cucumber beetle data (percent disk areas consumed) were arcsin square-root transformed prior to *t*-test analysis using the Sidak experimentwise correction for eight tests ($P \leq 0.006$) and *F*-tests on differences (treated–control) in feeding to examine leaf location effects. For the field study, a Wilcoxon rank-sum test was used to assess treatment differences at the time when insect densities on fungal-treated and control plants were highest (2 weeks after infection for beetles, 6 weeks for aphids.

Results

Induction of peroxidase enzymes and phenolics by *C. cucumerinum*

Peroxidase activity was significantly induced in cucumber plants inoculated with *C. cucumerinum* in both local, symptom-bearing and systemic, symptom-free leaves by the 4th day after infection (three-way ANOVA, $F_{15,41} = 2.58$, $P = 0.008$, $R^2 = 0.49$; treatment effect $F = 20.50$, $P < 0.0001$; day effect $F = 2.84$, $P = 0.05$; leaf location effect $F = 291.6$, $P < 0.0001$; treatment × day interaction $F = 2.72$, $P = 0.06$). Inoculation led to significant increases in activity earlier in local leaves (Fig. 1A) than in systemic leaves (Fig. 1B).

Folin-reactive phenolic content differed significantly in cucumber leaves ($F_{15,48} = 3.00$, $P = 0.002$, $R^2 = 0.48$) by day of harvest ($F = 4.78$, $P = 0.005$), leaf position ($F = 9.66$, $P = 0.003$) and the date × leaf position interaction ($F = 4.06$, $P = 0.01$) but not according to *C. cucumerinum* treatment ($F = 0.05$, $P > 0.1$) and no increases in phenolics occurred in local leaves after inoculation (Fig. 2A). However, infection significantly enhanced phenolic levels in systemic leaves 3 days after infection (Fig. 2B). A second experiment confirmed increased concentrations in systemic but not local leaves of inoculated plants after 3 days (mean ± SE: 13.7 ± 0.685 mg/ml for systemic leaves from infected plants, 10.9 ± 0.339 mg/ml for mature control leaves, 11.3 ± 1.51 mg/ml for local infected leaves, 9.95 ± 0.498 mg/ml for young controls; $F_{3,16} = 10.43$, $P = 0.0005$, $R^2 = 0.66$, treatment effect $F = 17.91$, $P = 0.0006$).

SAR to fungal anthracnose

In two out of three replicates, smaller and fewer *C. orbiculare* lesions developed on systemic leaves from plants that had received *C. cucumerinum* inoculation than on leaves from control plants (grand means ± SE for all three trials; lesion area: 10.0 ± 1.32 inoculated plants,

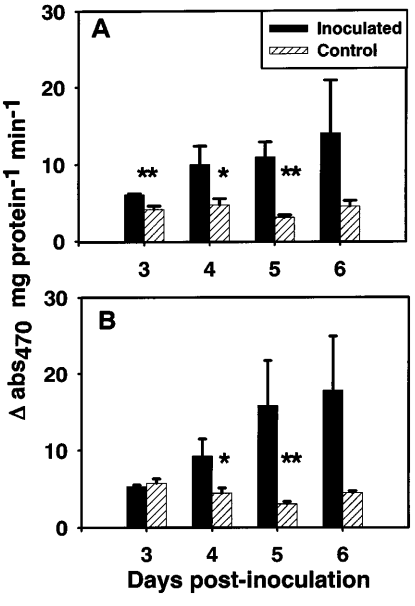

Fig. 1 Peroxidase enzyme activity (±SE) 3–6 days after inoculation with *Cladosporium cucumerinum* in local (**A**) or systemic leaves (**B**) (*abs* absorbance; *asterisks* denote significant one-way *F*-tests on the effect of treatment: $*P < 0.05$, $**P < 0.01$)

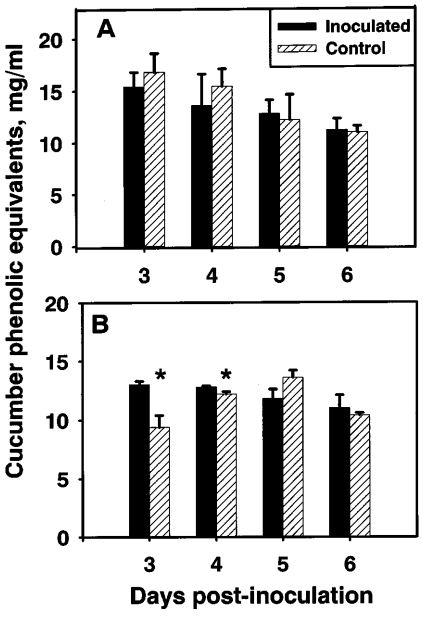

Fig. 2 Total phenolics in cucumber (±SE) 3–6 days after inoculation with *C. cucumerinum* in local (**A**) or systemic leaves (**B**) (*asterisks* as in Fig. 1)

14.8 ± 1.16 controls; lesion number: 22.0 ± 2.9 inoculated, 29.4 ± 3.78 controls; $P < 0.01$, $R^2 > 0.63$ for lesion area and number F tests on individual trials).

Beetle preferences on leaf disks

Spotted cucumber beetles did not remove differing areas from infected versus control disks taken from either local or systemic leaves, with the exception of local leaves 3 days after infection (Fig. 3) ($t = 13.64$, $P = 0.005$). Beetles tended to feed more on disks from partially necrotic local leaves of inoculated plants than on disks from young control leaves, while disks from mature control leaves tended to receive more damage than disks from systemic leaves of infected plants (Fig. 3) ($F_{7,19} = 3.01$, $P = 0.03$, $R^2 = 0.53$, leaf location effect $F = 15.44$, $P = 0.0009$).

Beetle and aphid no-choice tests

In two trials, spotted cucumber beetles consumed 30–45% more leaf area on local leaves bearing necrotic symptoms of *C. cucumerinum* than on young control leaves, but did not remove more leaf area from systemic, non-symptomatic leaves from infected plants versus mature control leaves (Fig. 4A). Leaf position significantly affected feeding in the second experiment (Fig. 4A, right pairs of bars) ($F_{2,16} = 5.37$, $P = 0.02$, $R^2 = 0.40$; leaf location effect $F = 5.07$, $P = 0.04$),

Melon aphids produced about 50% fewer offspring when confined on symptomatic local leaves versus young uninfected leaves, but showed no such difference for systemic leaves versus controls in the first trial (Fig. 4B, left pairs of bars). In a second trial, aphids produced significantly more offspring on local leaves, with once again no significant difference in systemic leaves (Fig. 4B, right pairs of bars). Leaf position did not significantly affect reproduction in this trial ($F_{3,16} = 9.99$, $P = 0.0006$, $R^2 = 0.65$; leaf location effect $F = 3.09$, $P = 0.10$). Local leaves used in the first trial were in many cases almost completely necrotic, while those harvested for the second trial were more comparable to leaves used for beetle bioassays, with no more than 50% necrotic leaf area.

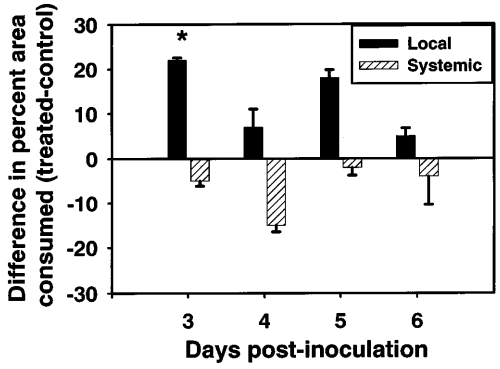

Fig. 3 Consumption of leaf disks by spotted cucumber beetles in dual-choice assays. Percent difference in consumption (±SE) of disks from local infected leaves relative to young control leaves, and of disks from systemic infected leaves relative to mature control leaves. *Asterisk* denotes a significant *t*-test ($P < 0.05$) on the difference in percent area consumed on treatment and control leaf disks

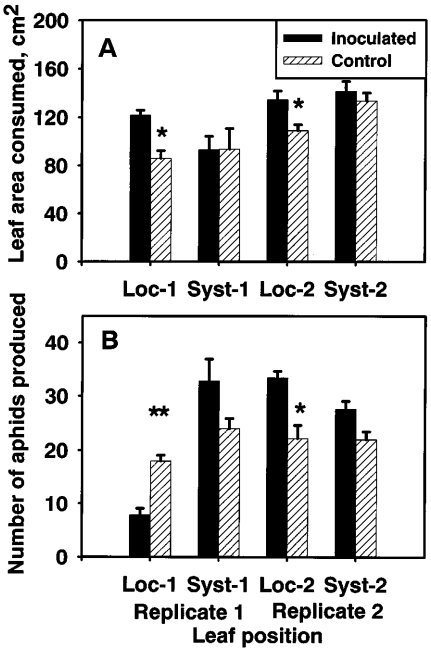

Fig. 4 Feeding by spotted cucumber beetles (**A**) or production of nymphs by apterous melon aphids (**B**) (±SE) on detached leaves from *C. cucumerinum*-inoculated or control plants. *Asterisks* denote significant one-way ANOVAs comparing treatments (*$P < 0.05$, **$P < 0.01$)

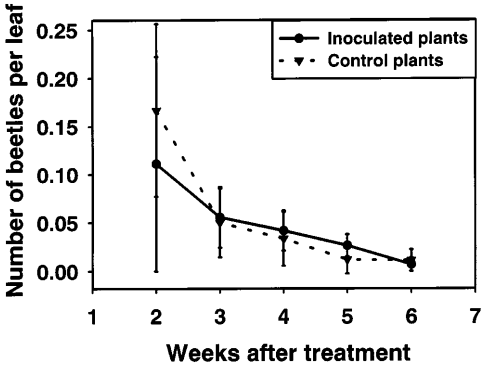

Fig. 5 Densities of cucumber beetles on field plants inoculated with *C. cucumerinum* and on control plants over 5 weeks (±SE)

Impacts of fungus on field exploitation

Spotted and striped cucumber beetle densities did not differ between inoculated and control plants 2 weeks after treatment (Fig. 5) (Wilcoxon rank-sum test, $Z = 1.08$, $P > 0.10$, $n = 22$ plants) and density declined over time in both treatments. Melon aphids did not appear until the 4th week after treatment, and densities

did not differ between fungus-infected and control plants 6 weeks after treatment ($Z = -0.777$, $P > 0.10$, $n = 22$ plants) (data not shown).

Discussion

Induction of peroxidase enzymes and phenolics in cucumber after *C. cucumerinum* infection varied according to leaf position and over time. Consistent with past results (Hammerschmidt and Yang-Cashman 1995), peroxidase enzyme activity in cucumber increased plant-wide after infection by a leaf necrosis-inducing fungal pathogen. Induction occurred earlier in local leaves, but did not differ spatially by 5 days after infection, the time point at which leaves on separate plants were harvested for no-choice bioassays with insects. In gel electrophoresis studies (P.J. Moran, unpublished data), peroxidase induction after *C. cucumerinum* infection involved increases in constitutive anionic isozymes, rather than the appearance of new isozymes (Smith and Hammerschmidt 1988; Svalheim and Robertsen 1990) that could have served as ecological markers of cross-effects of infection on pathogens and herbivores. Peroxidase induction was concurrent with induction of SAR against *C. orbiculare*, consistent with other studies (Hammerschmidt et al. 1976).

Three days after *C. cucumerinum* inoculation, phenolic induction occurred on systemic leaves of cucumber, but the response then gradually disappeared, in contrast to peroxidase enzymes. This trend agrees with Cherif et al. (1994) who found elevated phenolics in cucumber roots 6 days after *Pythium* spp. fungal infection but not on subsequent days, while peroxidase induction occurred over a longer time frame. Because extraction procedures were similar in the *Pythium* work and this study, phenolic extracts from *C. cucumerinum*-inoculated plants likely contained phytoalexins with antimicrobial activity against this same pathogen (Cherif et al. 1994), one of which was recently identified as a methyl ester of coumaric acid (Daayf et al. 1997). Phenolics mirrored peroxidases in not differing between leaf positions 5 days after inoculation, but both responses may have contributed to necrotic symptom development on local leaves (Nicholson and Hammerschmidt 1992), and the presence of symptoms determined whether or not cross-effects of infection on insects occurred at that time point.

Spotted cucumber beetles tended to prefer disks from partially necrotic local cucumber leaves over young controls, particularly 3 days after *C. cucumerinum* infection, and beetles fed more on local leaves than controls harvested 5 days after infection in no-choice tests. Consistent with these findings, leaf beetle larvae exploiting *Rumex obtusifolia* fed more on infected leaves bearing lesions than on healthy leaves (Hatcher et al. 1994, 1995). Insect feeding is a popular indicator of the ecological cross-effects of infection (see Table 1) but increases can be misleading: reductions in leaf beetle larval growth and adult beetle reproduction occurred on infected *Rumex* plants in spite of increased larval feeding, for example (Hatcher et al. 1994). Carbohydrate content and composition differed in areas around necrotic lesions compared to healthy tissue, and nitrogen decreased (Hatcher et al. 1995). These types of nutrient changes may be more important than antipathogenic peroxidases and phenolics in mediating cross-effects of infection on herbivores, and could have resulted in compensatory feeding in infected Straight 8 cucumber leaves. The dual-choice results with spotted cucumber beetles suggest that changes in herbivory arising from infection were relatively stable and were not tied to the timing of peroxidase and phenolic induction. Unlike the case of soybeans (Kogan and Fischer 1991), phenolic induction in systemic cucumber leaves did not reduce herbivory.

Melon aphid reproduction declined in one trial on necrotic versus young control leaves, and increased in a second experiment that was more comparable to trials with cucumber beetles. Consistent with these latter data, peach aphid distributions on viral-infected plants reflect enhanced feeding cues or host suitability on symptomatic leaves (Baker 1960). Necrosis after pathogen infection represents accelerated senescence, and on healthy plants, numerous aphid species prefer and perform better on senescing leaves (Hammond and Hardy 1988; Barbosa 1991). As with cucumber beetles, peroxidase and systemic phenolic induction did not mediate increased resistance to melon aphids. In contrast to beetles, cross-effects of *C. cucumerinum* on aphids may have been more dynamic than the chemical responses, since the two no-choice trials together suggest early benefits on partially necrotic leaves, followed by costs when necrosis becomes more advanced.

Preferences and increased feeding or reproduction by cucumber beetles and melon aphids did not translate to influences on insect densities on whole *C. cucumerinum*-infected plants in the field, in contrast to preferences by striped cucumber beetles for whole caged cucumber seedlings infected with a necrosis-causing virus (Apriyanto and Potter 1990) and increased alate aphid movement to virus-infected sugarbeets (Macias and Mink 1969). The significant dependence of cross-effects on leaf position and in some cases time may have contributed to the lack of field effects, since in the field experiment plants were not examined with the same degree of spatial resolution as greenhouse studies, and were not sampled until 2 weeks after inoculation. The more complex array of choices available to herbivores in common-garden plantings than in bioassay cages may have reduced the ecological importance of cross-effects in this system. Other field designs such as randomized complete blocks might have revealed insect choices more clearly, albeit with reduced relevance to more random natural ecosystems.

The field results notwithstanding, this study has demonstrated cucumber to be a good model for studying the ecological significance of pathogen infection on in-

sects. Both chemical and physical plant factors, and predators and parasitoids may influence herbivore distributions within plants (Root 1973; Jermy 1988; Schultz 1992; Woods et al. 1996). Differences between local leaves receiving inoculum and systemic leaves not subject to the same degree of impact could influence the survival of herbivores (Barbosa 1991) since both herbivores and their enemies can respond to plant characteristics. If behavior at one or more trophic levels is altered by plant pathogen infection, community-wide effects are possible.

Acknowledgements I thank Jack Schultz and Don Cipollini for their contributions to this work. *C. cucumerinum* cultures were provided by Dr. R. Hammerschmidt, Michigan State University. *C. orbiculare* cultures were provided by Dr. J. Kuc, University of Kentucky. Melon aphids were provided by Dr. T. Perring, University of California, Davis. Comments by Ahnya Redman and two anonymous reviewers substantially improved the manuscript. This work was supported by NSF grant DEB 9420013 to J. Schultz.

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