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Effect of Geminivirus Infection and *Bemisia* Infestation on Accumulation of Pathogenesis-Related Proteins in Tomato

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The whitefly, *Bemisia tabaci* biotype B, has been shown to cause pathogenesis-related (PR) proteins to accumulate in plants as a result of direct feeding, but their specific role in plant defensive systems is unclear. Our objective was to compare accumulation of tomato PR proteins (β-1,3-glucanase, chitinase, peroxidase, P2 and P4) in response to whitefly, with or without tomato mottle virus (ToMoV) infection. Tomato PR protein response was measured over time in plants divided into three treatments: uninfected controls (with or without whiteflies) and plants infested with viruliferous (ToMoV) whiteflies. Five- to six-leaf plants were infested with ~5 adult whitefly per leaf. Plants were sampled prior to whitefly infestation and at 14, 28, 42, and 56 days. By 56 days, plants infested with viruliferous whiteflies had significantly more eggs (2.5-fold) and nymphs (4.5-fold) than plants with nonviruliferous whiteflies. A significant increase in the enzymatic activity of all measured PR proteins, as compared to control plants, was only seen in viruliferous whitefly-infested plants. No significant difference was observed in enzyme activities between the uninfected control plants either with or without whiteflies. The greatest differences for all PR proteins assayed were observed 42 days after treatment initiation. Protein blot analyses showed that the differences in PR protein activities among the treatments were due to changes in specific enzyme levels within the plant and were associated with concomitant increases in levels of P2 and P4 PR proteins. Under our experimental conditions, it is clear that PR protein response is much more intense when it is attacked by whiteflies carrying ToMoV than by whitefly alone. Arch. Insect Biochem. Physiol. 49:203–214, 2002.

KEYWORDS: tomato mottle virus; *Bemisia tabaci* biotype B; *Bemisia argentifolii*; plant defense; induced response

INTRODUCTION

Increased whitefly-related crop damage has been attributed to the establishment of a new biotype (B) that was subsequently described as a new species, *Bemisia argentifolii* Bellows & Perring (Perring et al., 1993; Bellows et al., 1994). It is not known whether or not these two pests are biotypes of *Bemisia tabaci* or a species complex (Brown et al., 1995); however, the B biotype has an expanded host range, increased intercrop mobility, and enhanced ability to develop insecticide resistance. Another disturbing factor is that the identification of the B biotype is associated with the appearance of new crop disorders and virus diseases. In Florida, the appearance of the B biotype

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is linked to the appearance of a silverleaf disorder of squash (Kring et al., 1991; Schuster et al., 1991) and an irregular ripening disorder of tomato (Schuster et al., 1990). An additional problem associated with the B biotype is the increase of whitefly-vectored geminiviruses including golden bean mosaic, tomato mottle (ToMoV), and tomato yellow leaf curl virus (Polston and Anderson, 1997). The vectoring of these viruses increases the economic impact of the whitefly on vegetable production by reducing the economic threshold to a level of one whitefly per plant, which results in a zero tolerance for this pest. Currently, this zero tolerance relies heavily on chemical control, which may result in increased tolerance or development of insecticide resistance, further stressing the economic inputs required to sustain profitability.

Many investigations on biologically based mechanisms for controlling damage to crops by insects and pathogens have focused on plant defense mechanisms. Plants are known to have defense mechanisms that aid in protecting them from pathogen and/or insect attack. A class of proteins initially discovered because of their apparently unique synthesis in response to pathogen attack have been termed pathogenesis-related proteins and are thought to play a role in plant defense (van Loon, 1999). The term pathogenesis-related (PR) proteins was coined in 1980 for proteins coded for by the host plant, but induced only by pathogens, pests, or other stress-related situations (Antoniw and White, 1980). Under this broad definition, any host protein induced by any type of infectious agent or comparable condition would qualify as a PR protein. PR proteins are known to be expressed in response to various external stimuli, including pathogens, wounding, chemical elicitors, hormones, and UV-light (van Loon, 1985; Brederode et al., 1991; Inbar et al., 1998). In addition to pathogens, PR protein synthesis is also induced by attack from parasitic nematodes, phytophagous insects and herbivores (van Loon et al., 1994).

PR proteins are divided into at least 11 families representing β-1,3-glucanases, chitinases, thaumatin, and osmotin-like proteins, proteinase inhibitors, proteinases, peroxidases, ribonuclease-like proteins, or proteins of unknown function (van Loon, 1999). PR protein production can be modulated in response to different stimuli. In fact, PR protein accumulation varies among the different PR families and members of families depending on the type of stimuli and the host plant variety. Despite a continually growing knowledge about the structure and activity of PR proteins, their involvement in plant defense is not completely understood. In tomato plants, whiteflies alone have been shown to cause specific PR proteins (β-1,3-glucanase, chitinase, P2, and P4) to accumulate (Mayer et al., 1996). However, there are no reports on the response of tomato PR proteins to ToMoV, a whitefly vectored geminivirus.

In tomato, it is unclear how the level of PR protein accumulation in response to whitefly infestation compares to accumulation when whitefly vector a plant virus. Experiments were conducted to answer this question. Comparison of whitefly and whitefly/virus complex accumulation of tomato PR proteins and influence on whitefly fecundity are presented. It is the first report of tomato PR protein response to ToMoV and of virus-stimulated egg production on tomato by an insect vector.

MATERIALS AND METHODS

Insects

Adult male and female *B. tabaci* biotype B were obtained from laboratory colonies maintained by the U.S. Horticultural Research Laboratory, Ft. Pierce, FL. Whiteflies used in these experiments were originally obtained from Dr. Lance Osborne, University of Florida in Apopka, FL, and have been maintained on dwarf cherry tomato (*Lycopersicon esculentum* cv. Florida Lanai) since 1996 by serial transfer. In 1997, a ToMoV whitefly colony was established by obtaining tomato plants infected with ToMoV from Dr. Philip Stansly, University of Florida in Immokalee, FL, and infesting with whitefly from the healthy colony. Whitefly biotyping was based on RAPD PCR analysis using primers developed by De Barro and Driver (1997). Nonviruli-

ferous and viruliferous whitefly colonies were housed separately in screened Plexiglas cages located in separate greenhouses. Whiteflies from the viruliferous colony were confirmed to be infected with ToMoV prior to infestation by PCR analysis (Sinisterra et al., 1999).

Experiment Design

Tomato seeds (*Lycopersicon esculentum* cv. Florida Lanai) were directly seeded into speedling trays and allowed to germinate. Plants with 5 to 6 fully expanded leaves were planted individually in 4" pots with time-release Osmocote 14-14-14 fertilizer (Scotts-Siera Horticultural Products Company, Marysville, OH) when the trial was initiated. Each treatment group was contained in a large screened plexiglas cage and maintained separately in Environmental Growth Chambers (Environmental Growth Chambers, Chagrin Falls, OH) at 25 ± 1°C under a L/D, 16:8 photoperiod. Treatments included an untreated control, whiteflies from the healthy colony, and whiteflies from the ToMoV infected colony. Five adult whitefly per leaf (30 per plant) of unknown age or sex were released into their respective treatment cages and allowed to infest plants naturally. For the next five days, whiteflies were counted on 20 leaves randomly chosen from the 35 plants in each treatment regime. If the mean was lower than 5 whitefly per leaf, then whiteflies were supplemented as needed to correct for natural mortality and maintain the initial infestation rate of 5 per leaf. Plant samples were taken immediately prior to whitefly infestation to establish baselines for protein and enzyme activity. Subsequent plant samples were taken at 14, 28, 42, and 56 days post-whitefly infestation. The results are reported as seven replications within the experiment and experiments were repeated three times.

Immature whitefly counts were taken from no. 4 size cork borer plugs (~0.4 cm^2) and samples were taken from the 4th through 8th tomato leaflet (5 per plant) from the terminal of each plant (7 plants) on day 56 post-whitefly infestation. Counts included whitefly eggs, nymphs (crawler through early fourth instar), and red-eye (late fourth instar) pupae stages. The same number (30 per plant) of whiteflies was introduced into each treatment at the beginning of the trial. All 7 plants from the ToMoV treatment exhibited severe virus symptoms.

Plant Sample Preparation

Plant samples consisted of pooling the first three fully expanded leaves from the terminal of each plant. Fresh leaf samples were cut and immediately frozen with liquid nitrogen. Leaf samples were ground in liquid nitrogen using a mortar and pestle and weighed. Ground leaf samples were poured into centrifuge tubes containing 0.6 g of hydrated polyvinylpolypyrrolidone (Sigma, St. Louis, MO). Sodium phosphate buffer (0.1 M; pH 7.4) was added to each centrifuge tube at 5 volumes of buffer per g of leaf material. The tubes were capped and mixed for 20 min at 4°C and then centrifuged at 26,817*g* for 20 min. The supernatant was filtered through a layer of Miracloth (CalBiochem, La Jolla, CA) into dialysis tubing with a molecular weight cutoff of 6,000–8,000 daltons. Samples were dialyzed overnight against 10 mM sodium phosphate buffer (7.4 pH) at 4°C and then stored at –80°C until ready for protein and enzyme analysis.

Determination of Protein Concentration and Enzyme Activity

Protein concentrations were determined using bovine serum albumin as the standard using the Bradford method (1976). Unknown samples and standard curves were read at 595 nm using an Optimax™ microtiter plate reader (Molecular Devices Corporation, Sunnyvale, CA). Chitinase, peroxidase, and β-1,3-glucanase levels were measured as previously described by Mayer et al. (1996).

Protein Blot

Ten micrograms of proteins were separated by using 12% SDS-PAGE (Laemmli, 1970). Electro-

phoresed proteins were blotted to Hybond P membranes (Amersham Pharmacia Biotech Inc., Piscataway, NJ) using a Trans-Blot SD semi-dry electrophoretic Transfer Cell (BioRad Laboratories, Hercules, CA). Immunodetection of the specific PR proteins was performed using the ECL+Plus chemiluminescent detection kit and protocols (Amersham Pharmacia Biotech, Inc.). Primary antibodies were incubated with the membranes at a 1:1,000 dilution and the secondary antibody was diluted 1:5,000. Primary antibodies were developed for tomato PR proteins (26 kDa acidic chitinase, β-1,3-glucanase, P2 and P4 proteins) obtained from Dr. P.J.G.M. De Wit. The antibodies and proteins they were obtained from have been described in Joosten and De Wit (1989) and Joosten et al. (1990). Chemiluminescent results were visualized and quantitated using a Kodak IS440CF (Rochester, NY) imaging station.

ELISA

All plant samples were screened for geminiviruses using Pathoscreen 3F7 ELISA test kit and protocol by Agdia Incorporated (Elkhart, IN).

Statistical Analysis

Data were analyzed using the one-way analysis of variance option of the general linear models (GLM) procedure and the means were compared using Tukey's Studentized Range (HSD) Test (SAS Institute, Inc., 1998). Statistical level of significance was $P < 0.05$. Correlation analysis (SAS) was used to determine relationships between enzyme activity and geminivirus infection as determined by ELISA.

RESULTS

Whitefly Development and Plant Responses to Virus

Virus-infected whiteflies used in the experiment were taken from a population shown to harbor ToMoV using a PCR-based assay (unreported data). Although the initial whitefly count on the tomato plants was maintained at 5 per leaf for the first 5 days of the experiment, by 56 days post-whitefly infestation, there was a significant difference ($P < 0.05$) in the number of eggs and nymphs (crawler through early fourth instar) per plant between the nonviruliferous and viruliferous whiteflies (Table 1). Plants infested with viruliferous whiteflies consistently had 2.5-fold more eggs and 4.5-fold more nymphs than the plants with the nonviruliferous whiteflies ($P < 0.05$). By 28 days post-whitefly infestation, a faint mottle had begun to appear in plants infested with viruliferous whiteflies. By day 56, all plants displayed severe virus symptoms including chlorotic mottling and upward curling of leaflets and overall reduction in plant height.

Further characterization of the ToMoV infection was performed using ELISA of leaf extracts. Although minor virus symptoms began to appear by day 28 post-whitefly infestation, ELISA analysis for geminivirus was not sensitive enough to detect significantly higher levels of the ToMoV coat protein (Fig. 1). By 42 days post-whitefly infestation, the virus titer was double that from the previous date and was doubled again by 56 days post-whitefly infestation in the viruliferous whitefly-infested plants.

Chitinase, β-1,3-Glucanase, and Peroxidase Enzyme Activities

It was previously shown that whiteflies can cause total levels of chitinase, β-1,3-glucanase, and peroxidase activities to accumulate in tomato leaves (Mayer et al., 1996). A lower initial infestation level

TABLE 1. Comparison of the Mean Number of Immature Whitefly on Tomato 56 Days Post-Infestation With Nonviruliferous Whitefly and Whitefly Carrying Tomato Mottle Virus*

	Mean number (± SE) immature whitefly		
Whitefly treatment	Eggs	Nymphs	Red-eyes
Uninfested control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Nonviruliferous	18.6 ± 2.9b	2.4 ± 0.3a	0.8 ± 0.2b
Viruliferous	46.8 ± 4.2c	10.8 ± 1.7b	1.2 ± 0.3b

*Column means followed by the same letter are not significantly different ($P < 0.05$, HSD); sample size = 0.4 cm^2; $n = 35$ samples per treatment. Nymphs includes crawler through early fourth instar. Red-eyes include the nonfeeding portion of the late fourth nymphal instar found after apolysis has occurred when the pharate adult form is present, has red eyes and the yellow body pigment of the adult.

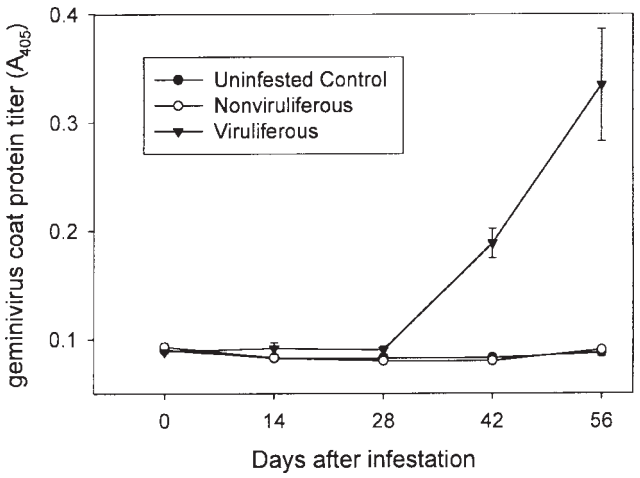


Fig. 1. ELISA detection of geminivirus coat protein in tomato plants over time. The same leaf extracts used for enzyme assays and Immunodetection analysis were used for ELISA based geminivirus detection. Thirty micrograms of protein for each sample was used and the protocols from the Pathoscreen kit were followed. Samples were quantitated by reading the absorbance at 405 nm using a microtiter plate spectrophotometer.

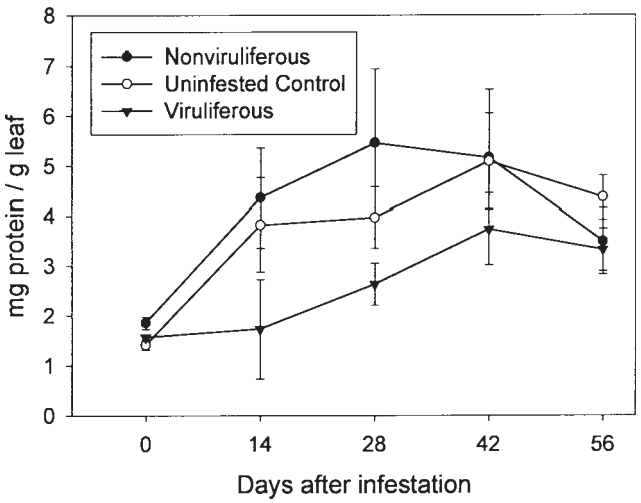


Fig. 2. Foliar protein in tomato exposed to ToMoV and whiteflies over time. Foliar protein was determined using the standard Bradford assay and Bradford reagent and protocols provided by BioRad (Hercules, CA). Values are Mean ± SE (n = 7).

than was used in that report was chosen for this work since the intent was to evaluate the effect of whitefly and ToMoV and a heavy initial whitefly infestation may have masked virus effects. Because there was a concern over how to normalize the enzyme assay data, either on a mg protein or g fresh weight basis, we compared the leaf protein content on a g fresh leaf weight basis. Figure 2 shows that virus-infected plants appeared to have a lower protein content between 14 and 28 days post infestation based on standard error. However, there was no statistical differences among the three treatments at any timepoint ($P < 0.05$). Because of the observed trend of lower protein in virus-infected leaves, enzyme results are presented both as a proportion of total leaf protein and as a proportion of total leaf mass. Only those differences that occur under both comparisons are reported.

In leaves of uninfected control plants (with or without whitefly), total chitinase enzyme activity declined while total β-1,3-glucanase, and peroxidase activity increased during plant development (Figs. 3–5). Leaves from plants challenged with viruliferous whiteflies showed an elevated level of all three enzymatic activities significantly higher than in uninfected control plants by day 42 ($P < 0.05$), and a significantly higher level of chitinase activity at day 28 ($P < 0.05$). Interestingly, none of the enzyme activities differed between the uninfected control plants, either with or without whiteflies at 56 days post infestation. This was predominantly due to a rise in plant PR protein enzymatic activities in control plants at 56 days, and not due to a significant reduction in any of the three measured enzymatic activities in the viruliferous whitefly challenged plants. As expected, increases in total leaf activity of each of the three enzymes was positively correlated with geminivirus coat protein ELISA results 42 days post-whitefly infestation (Table 2).

Protein Blot Analyses of PR Proteins in Response to Plant Treatment

Total leaf chitinase, β-1,3-glucanase, and peroxidase are a result of the activity of numerous isozymes and allozymes within each tissue. Analysis of quantitative changes in the abundance of PR proteins was performed by immunodetection of

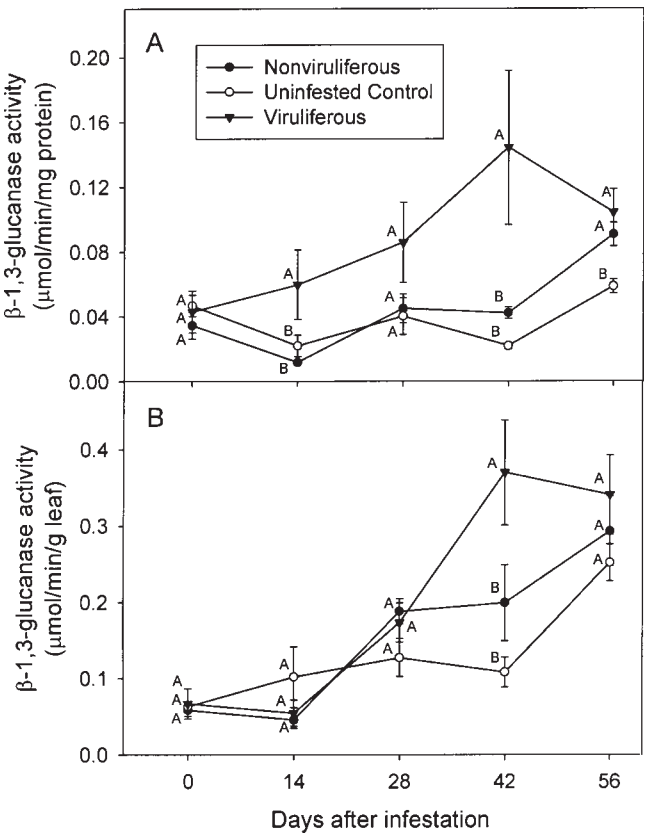


Fig. 3. Response of tomato leaf β-1,3-glucanase activity to whitefly/whitefly-ToMoV infestation. **A:** Foliar β-1,3-glucanase activity (per mg protein) in tomato exposed to ToMoV and whiteflies over time. **B:** Foliar β-1,3-glucanase activity (per g leaf) in tomato exposed to ToMoV and whiteflies over time. Means ± SE (n = 7) followed by the same letter are not significantly different ($P < 0.05$, HSD).

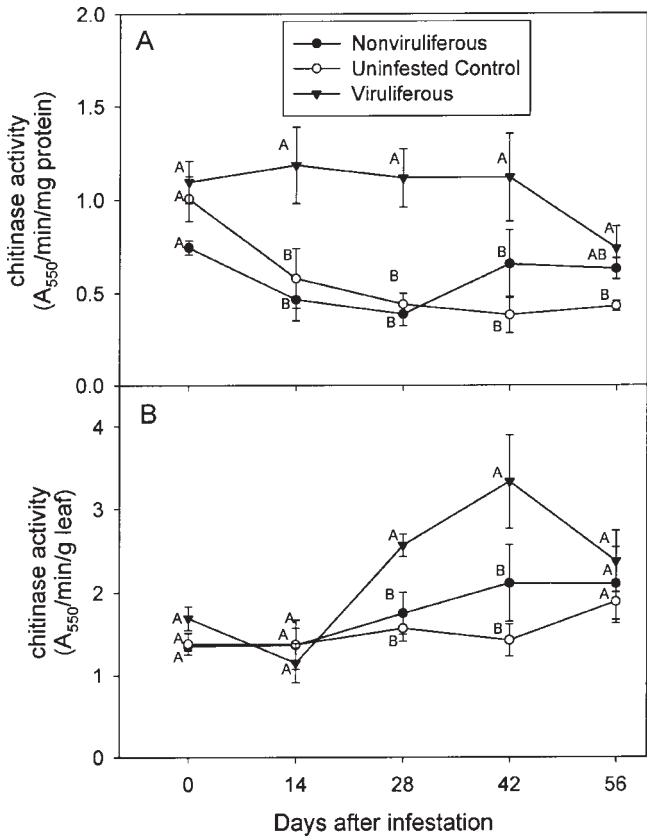


Fig. 4. Response of tomato leaf chitinase activity to whitefly/whitefly-ToMoV infestation. **A:** Foliar chitinase activity (per mg protein) in tomato exposed to ToMoV and whiteflies over time. **B:** Foliar chitinase activity (per g leaf) in tomato exposed to ToMoV and whiteflies over time. Means ± SE (n = 7) followed by the same letter are not significantly different ($P < 0.05$, HSD).

specific PR proteins separated by SDS-PAGE. These comparisons are shown for the pre-infestation and 42 days post-whitefly infestation samples (Fig. 6). These comparisons were performed for all sampling time points and results were always consistent with enzymatic data; however, to simplify the explanation, only data where significant differences were observed in enzymatic analysis are presented. Due to a high level of variation of enzyme activities and virus titer in the virus-infected plants, results were compared between viruliferous plants with a high level of induced PR protein enzyme activity (V2) and those with a lower level of enzyme induction (V1). The V1 plants had 1.9-, 1.8-, 2.1-, and 2.2-fold lower β-1,3-glucanase, chitinase, peroxidase, and virus titer than in the V2 plants, respectively.

One major chitinase with an M_r of 30 kD was present in all pre-infestation samples from control, nonviruliferous and viruliferous whitefly infested plants (Fig. 6B). All 42 days post-whitefly infestation samples had this same immunoreactive chitinase; however, it was much more abundant in the leaves of ToMoV-infected V1 and V2 plants as compared to the uninfested control plants and nonviruliferous whitefly-infested plants. Quantitation of chemiluminescent signal from chitinase immunodetection showed that chitinase in leaves of ToMoV-infected V1 and V2 plants was 6.9 and 9.5 times more abundant, respectively, than the un-

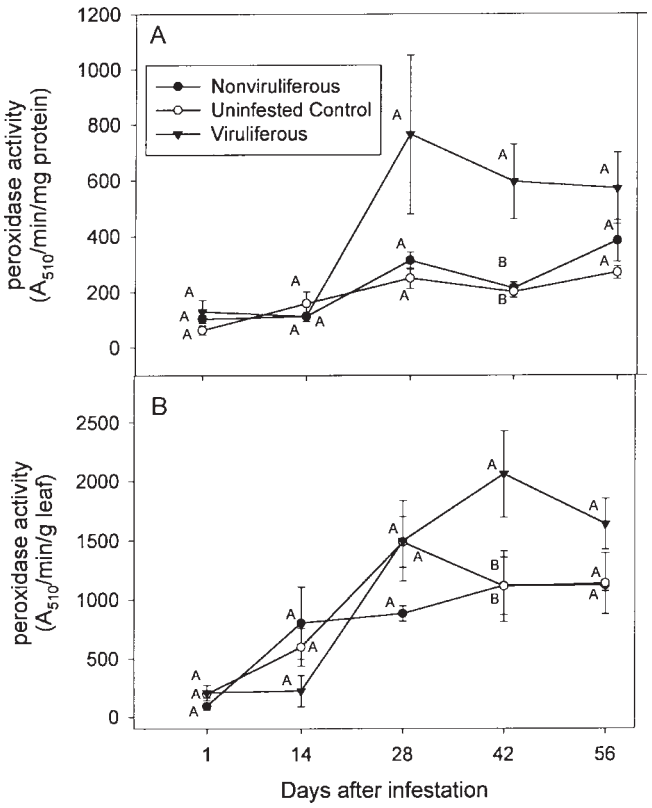


Fig. 5. Response of tomato leaf peroxidase activity to whitefly/whitefly-ToMoV infestation. **A:** Foliar peroxidase activity (mg protein) in tomato exposed to ToMoV and whiteflies over time. **B:** Foliar peroxidase activity (g leaf) in tomato exposed to ToMoV and whiteflies over time. Means ± SE (n = 7) followed by the same letter are not significantly different ($P < 0.05$, HSD).

infected control chitinase. Level of detection of this protein was not different in the nonviruliferous whitefly-infested plants as compared to the uninfested control plants.

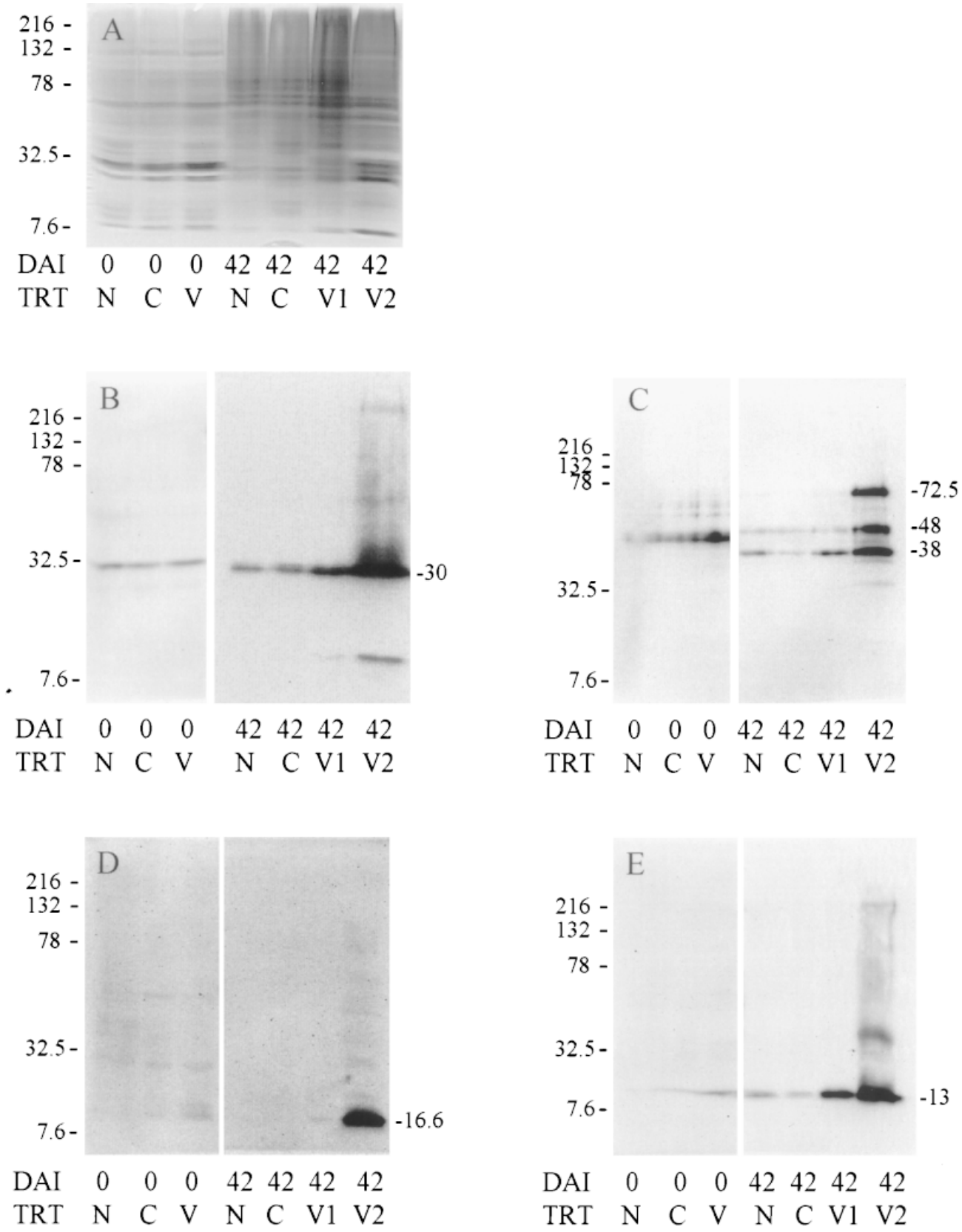
Antiserum against tomato β-1,3-glucanase also recognized proteins in all pre-infestation plants (Fig. 6C). The major protein had an M_r of ~45 kD. At 42 days post-whitefly infestation, a different set of β-1,3-glucanase-related proteins were recognized in control and treatment plants. Three proteins with M_r's of 38, 48, and 72.5 kD were detected in the uninfested control plants. The same proteins were visible in the nonviruliferous whitefly and viruliferous whitefly-infested plants. The 72.5 kD protein was 5 and 350 times more abundant in the V1 and V2 ToMoV infected plants, respectively, than in the leaves of the uninfested and whitefly infested controls. The 38- and 48-kD proteins were 33.8 and 13.5 times more abundant in the V2 plants as compared to uninfested control plants. In V1 plants, there was essentially no difference in the 48-kD protein level and a 3.5- and 8.4-fold increase in the 38-kD protein abundance compared to uninfested and whitefly infested control plants, respectively.

Additionally, immunodetection of two well-characterized 14 kD PR proteins (P2 and P4) previously reported in tomato (Joosten et al., 1990) was examined. The P2 and P4 proteins were chosen because their accumulation in response to fungal pathogen attack (Joosten et al., 1990) and

TABLE 2. Correlation Between Enzyme Activity and Geminivirus Titer as Determined by ELISA in Tomato Over Time[†]

Enzyme	Expression of enzyme activity	Days after infestation				
		0	14	28	42	56
	/mg protein					
Chitinase		–0.28 ns	0.30 ns	0.49*	0.55*	0.29 ns
β-1,3-glucanase		–0.07 ns	0.57**	0.39 ns	0.64**	0.33 ns
Peroxidase		–0.06 ns	0.27 ns	0.36 ns	0.73***	0.51*
	/g leaf					
Chitinase		–0.09 ns	0.21 ns	0.24 ns	0.41*	0.08 ns
β-1,3-glucanase		0.01 ns	0.24 ns	0.16 ns	0.51*	0.16 ns
Peroxidase		0.03 ns	0.04 ns	0.17 ns	0.47*	0.53*

[†]Virus titers (Fig. 1) are correlated with enzyme data (Figs. 3–5). ns, not significant.
*$P < 0.05$
**$P < 0.01$
***$P < 0.001$.


A
216 -
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DAI 0 0 0 42 42 42 42
TRT N C V N C V1 V2
B
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216 -
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7.6 -
-72.5
-48
-38
DAI 0 0 0 42 42 42 42
TRT N C V N C V1 V2
D
216 -
132 -
78 -
32.5 -
7.6 -
-16.6
DAI 0 0 0 42 42 42 42
TRT N C V N C V1 V2
E
216 -
132 -
78 -
32.5 -
7.6 -
-13
DAI 0 0 0 42 42 42 42
TRT N C V N C V1 V2

Fig. 6

whitefly infestation (Mayer et al., 1996) have been well characterized. The P2 protein was only faintly detected in pre-infested samples (Fig. 6D). High level of accumulation of P2 was only observed in the V2 samples and faintly visible in the V1 sample at 42 days post-whitefly infestation. At this same time period, no P2 was visible in the nonviruliferous whitefly-infested or uninfested control plants. The relative detection of P4 was similar to that of P2, with the exception that the baseline level of P4 in pre-infested and uninfested control plants was slightly higher (Fig. 6E). At 42 days post-whitefly infestation, the highest level of P4 accumulation was in the V1 and V2 samples, and was 9.8 and 30 times more abundant than in controls, respectively.

DISCUSSION

The induction of PR proteins in response to specific types of abiotic and biotic stress or attack is a well-accepted phenomenon (for review see Datta and Muthukrishnan, 1999). However, the reason for the accumulation of these enzymes/proteins, and the mechanism by which specific stresses/attacks induce them, is still not well understood. It is apparent that the regulation of PR proteins is not a simplistic occurrence, and that many PR proteins are differentially regulated depending on the type of stress/attack that the plant is experiencing (Stout et al., 1998, 1999). Insect damage as well as pathogen attack can clearly induce PR proteins (Stout et al., 1994; Stout et al., 1995; Stout and Duffey, 1995). This work shows that the tomato PR protein response is much more intense when it is attacked by whiteflies carrying ToMoV than by whitefly alone; this is the first report of tomato PR protein responses to ToMoV. All measured PR proteins (β-1,3-glucanases, chitinases, peroxidases, P2, and P4) accumulated to a higher level in response to the presence of the whitefly-ToMoV complex at 42 days post infestation. This was not as apparent at day 56 by comparison of enzymatic activities (Figs. 3–5); however, at this time, the level of some PR protein activities in the leaves of control plants had risen, most likely as a result of plant developmental signals. Therefore, although PR protein activity rose in leaves of tomato as the plant matured, this rise occurred significantly earlier and at a higher level in virus-infected plants.

Interestingly, during the time when virus-infected plants had overall higher levels of PR proteins than either of the uninfected controls (with or without whiteflies), whitefly fecundity and nymph development was also significantly increased on plants infected with ToMoV as compared to the uninfected controls (Table 1). On tomato, whitefly developmental time from egg to adult ranges from 22 to 28 days (Coudriet et al., 1985), which would place immature whitefly stages counted at day 56 developing at the time when PR protein activity was the highest in the viruliferous treatment. Costa et al. (1991) found that whitefly progeny only survived better on virus-infected pumpkin compared to healthy pumpkin out of six virus-plant hosts evaluated, including tomato. However, all whiteflies used in these experiments

Fig. 6. Protein blot and immunodetection of PR proteins in tomato leaf samples. Twenty micrograms of total protein from each sample were separate by SDS-PAGE on 12% polyacrylamide gels and electroblotted to Hybond P membranes (Amersham Pharmacia, Buckinghamshire, England). Proteins were immunodetected using polyclonal antiserum to each of the proteins and the ECL+Plus chemiluminescent detection system from Amersham Pharmacia. **A:** Silver stain of total protein separated by SDS-PAGE. **B:** Immunodetection of tomato chitinase using a 1:1,000 dilution of chitinase antiserum. **C:** Immunodetection of tomato β-1,3-glucanase using a 1:1,000 dilution of β-1,3-glucanase antiserum. **D:** Immunodetection of tomato P2 PR protein using a 1:1,000 dilution of the P2 antiserum. **E:** Immunodetection of tomato P4 PR protein using a 1:1,000 dilution of the P4 antiserum. DAI, days after inoculation; TRT, plant treatment; N, plants infested with nonviruliferous whiteflies; C, control plants not infested with whiteflies; V, Plants infested with ToMoV viruliferous whiteflies; V1, extract from viruliferous whitefly infected plants with low level of PR enzyme activity induction; V2, extracts from viruliferous whitefly infected plants with high level of PR enzyme activity induction. Dashes along the side of each membrane represent the location of MW markers of 216, 132, 78, 32.5, and 7.6 kD.

had been maintained on pumpkin since 1983 and results did not take into consideration whitefly adaptation to a new host plant regardless of plant health status. This is the first report of a geminivirus increasing the fecundity of whiteflies on tomato and raises some interesting questions. Is the increased fecundity the result of a direct effect of the virus on whitefly egg production or is it a result of better performance on a virus-infected plant? Also, does the increased egg and nymph production result in more adult whitefly or is the increased fecundity necessary to keep the status quo? Studies are underway to answer these questions. The fact that the number of red-eyed nymphs (pupae stage) was not significantly different between the nonviruliferous and viruliferous whiteflies indicates that increased egg production occurred at some time after the viruliferous whiteflies were placed on healthy plants and that the whiteflies performed better due to changes in plant physiology. This could occur if ToMoV increased the nutritional value of the plant sap, which would subsequently be reflected in greater fecundity and insect numbers. This hypothesis remains to be tested.

Although the presence of ToMoV stimulates PR protein accumulation, we cannot determine if this increase was due directly to the virus or to the higher population of whiteflies on the plants infected with ToMoV, since at high levels of whitefly infestation, PR protein accumulation is stimulated (Mayer et al., 1996). The lack of significant changes in plant PR proteins in response to nonviruliferous whitefly infestation appears to contradict previous reported results (Mayer et al., 1996). However, lower levels of initial whitefly infestations and older plants were used in this study compared to the previous work. Since our data clearly show an elevation of β-1,3-glucanase and peroxidase activities in control plants as the plant matures, this increase may be masking whitefly-induced changes that were observed using younger plants and higher whitefly populations (Mayer et al., 1996). Although not significant, the trend for increased PR protein levels in nonviruliferous whitefly infested plants vs. uninfested control plants suggests that whitefly populations are building up to the levels needed to stimulate a larger scale plant defense response. Whatever the cause, the whitefly-ToMoV virus complex is a much more potent inducer of plant PR proteins than whitefly alone. Further studies are needed to characterize the whitefly-ToMoV virus complex and how that complex affects whitefly biology and the chemical nature of the induced response. An important question to answer is, do the whiteflies and some of the viral pathogens they vector work in concert with each other to ensure reproductive success?

The relationship of the whitefly, ToMoV, and the host plant is complex. Whitefly infestations of plants generally limit competition with other insect herbivores (Inbar et al., 1999) reserving the host plant for whiteflies. Because *B. tabaci* is attracted most strongly to yellow/green, followed in decreasing order by yellow, red, orange/red, dark green, and purple (Husain and Trehan, 1940), it is conceivable that the ensuing chlorosis observed on whitefly-infested plants could act as an attraction for other whiteflies in the field, some of which could be vectoring phytopathogens such as ToMoV. Likewise, ToMoV infected host plants exhibiting typical ToMoV symptoms that include either a bright yellow mosiac or a mottled, interveinal chlorosis may be more attractive to whitefly simply because of their stronger attraction to the yellow/green combination. What is not known is whether or not ToMoV-infected plants have similar effects on whitefly competing herbivores. Our data show that despite a virus-stimulated accumulation of the plant PR proteins, whitefly egg laying productivity and nymph development is higher on virus-infected plants. Determining how this impacts whitefly egg/nymph and competing herbivore development should be a priority.

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