Ingestion, transmission, and persistence of *Chino del tomate virus* (CdTV), a New World begomovirus, by Old and New World biotypes of the whitefly vector *Bemisia tabaci*

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Summary

Two whitefly biotypes of *Bemisia tabaci*, from either the Eastern or Western Hemisphere, respectively, were compared with respect to their competency to ingest and their efficiency to transmit the New World begomovirus, *Chino del tomate virus* (CdTV). The AZ A biotype of *B. tabaci* originates from the arid southwestern USA and northwestern Mexico, while the B biotype has an origin in the Middle East or Northern Africa. The ability of these two vector biotypes to ingest and subsequently to transmit CdTV were evaluated for an acquisition-access period (AAP) that ranged from 0 to 72 h, followed by a 48 h inoculation-access period (IAP). Individual adult whiteflies were monitored for CdTV ingestion using polymerase chain reaction (PCR) to detect the viral coat protein gene (AV1 ORF), and transmission efficiency (frequency) was determined by allowing potentially viruliferous whiteflies access to tomato seedlings following each experimental AAP. PCR results for individual adult whiteflies indicated that CdTV was ingested from infected tomato plants by both biotypes 93% of the time. Transmission frequencies by both vector biotypes increased with longer AAPs. However, the AZ A biotype transmitted CdTV 50% of the time, compared to only 27% for the B biotype. Evidence that virus was ingested with equal competency by the A and B biotypes confirmed that both vectors were capable of ingesting CdTV from tomato at the same frequency, even when the AAP was 0.5 h. Consequently, either the acquisition and/or transmission stages of the pathway, rather than ingestion competency, were responsible for differences in vector-mediated transmissibility. Detection frequency of CdTV, after 48 h AAP, by PCR in single females of AZ B biotype was significantly higher than males.

Introduction

Whitefly-transmitted geminiviruses, or Begomoviruses (Family: Geminiviridae), have long been known to cause damaging diseases of crop plants in subtropical and tropical locales, worldwide (Bird & Maramorosch, 1978; Costa, 1976; Muniyappa, 1980; Varma, 1963). In the 1980s, begomoviruses were recognised as important pathogens of many herbaceous and certain woody crops grown in temperate regions that border subtropical zones (Brown & Bird, 1992), and, most recently, have been recognised as emergent pathogens in agroecosystems in mild climatic zones throughout the world (Brown, 1994, 2001a).

Begomoviruses have either a bipartite or monopartite, circular, single-stranded DNA genome of approximately 5.2 (bipartite) or 2.8 (monopartite) kilobases, respectively (Lazarowitz, 1992). The coat protein (CP) (AV1 ORF) is the most highly conserved among begomoviral-encoded proteins, owing to its multifunctional nature. The CP is essential

for virion assembly, for host range determination in some instances (Ingham *et al.*, 1995; Jeffrey *et al.*, 1996), and is the only viral determinant of whitefly-mediated transmission to be identified to date (Briddon *et al.*, 1990). Begomoviruses are transmitted exclusively by the whitefly, *Bemisia tabaci* (Gennadius) in a circulative, persistent manner (Briddon *et al.*, 1990; Brown, 1994; Harrison, 1985). These collective properties account for the recognition of the CP as the most highly conserved viral gene, and its acceptance by the ICTV for establishing provisional identification of species and strains (Mayo & Pringle, 1998).

Two distinct biological biotypes of *B. tabaci* were initially recognized in Puerto Rico as 'races' for which host range and life history traits could be distinguished (Bird, 1957; Bird & Maramorosch, 1978). The recent recognition of distinct biological biotypes, worldwide (Bird & Maromorosch, 1978; Brown & Bird, 1992; Burban *et al.*, 1992; Costa & Brown, 1991; Costa *et al.*, 1993; Frohlich *et al.*, 1999), their potential to influence disease epidemiology and ultimately

evolution of begomoviruses, has resulted in the need to understand the biological and genetic basis for such differences (Brown, 2001*b*). Recent studies have provided evidence of notable genetic variability within the species, worldwide, and have led to the suggestion that *B. tabaci* is a species complex, or a collection of sibling species (Brown *et al.*, 1995*a*; De Barro *et al.*, 2000; Frohlich *et al.*, 1999; Gawel & Bartlett, 1993; Kirk *et al.*, 2000).

Because most *B. tabaci* studied to date are quite polyphagous, subtle distinctions in host range have proven more difficult to ascertain for those of agricultural importance. Moreover, the basis for differences in transmission competency that has been shown for certain well-studied *B. tabaci*-begomovirus complexes is poorly understood, as is the outcome of potential differential transmission to the evolution of begomoviruses and their co-evolution with their whitefly vector. However, whether overall transmission competency is primarily, or collectively, influenced at the level of interactions between whitefly-plant host, virus-host, and/or virus-vector, or moreover, at specific stages in the transmission pathway, including ingestion, acquisition, or egestion, is not known.

In this study, we investigated the competency of the transmission pathway for two biotypes of *B. tabaci* from the Old and New World, respectively, and *Chino del tomate virus*, (CdTV) (Brown & Nelson, 1988; Brown *et al.*, 2000*a*), a tomato-infecting begomovirus from the New World. While both whitefly vectors readily colonise tomato, the New World AZ A biotype of *B. tabaci* originated from irrigated agroecosystems in the southwestern US and northwestern Mexico, and the B biotype originated from the Middle East or Northern Africa. Here, we report experiments in which competency for virus ingestion and transmission, and persistence of the New World CdTV in the vector were examined over a range of acquisition-access periods for two biotypes of *B. tabaci* of distinct, predicted evolutionary lineages.

Materials and Methods

Whitefly vector colonies

Two biological biotypes of *B. tabaci*, the Arizona A (AZ A biotype) and B (AZ B biotype), were compared as vectors of CdTV. Colonies were reared on cotton plants *Gossypium hirsutum*, 'Delta Pine and Land Company 70' (DP 70) in physically separated growth rooms as described (Idris & Brown, 1998). The AZ A biotype was collected from cotton *Gossypium hirsutum* fields in Phoenix, AZ in 1981 and has been maintained in a colony on cotton or pumpkin *Cucurbita maxima* in a growth room at the University of Arizona, Tucson, AZ (USA) campus since that time. The B biotype colony originated from

naturally-infested poinsettia plants in a greenhouse at the University of Arizona in 1987, and has been identified as an exotic introduction from the Old World (Brown *et al.*, 1995*a*; Frohlich *et al.*, 1999). The AZ A and B biotypes differ from one another with respect to differences in several important biological characteristics, including host range and fecundity, among others, and are readily distinguishable from each other and from several other well-studied biological biotypes of *B. tabaci* based on distinct, genetic polymorphisms (Bedford *et al.*, 1994; Brown *et al.*, 1995*b*, 2000*b*, 1995*a*; Frohlich *et al.*, 1999).

A colony of *Trialeurodes vaporariorum* (West), the greenhouse whitefly, was established on tobacco *Nicotiana tabacum* cv Samsun plants and used as a non-vector control. *T. vaporariorum* is a polyphagous whitefly that colonizes many of the same herbaceous species as *B. tabaci*, and it has been shown capable of ingesting, but not transmitting begomoviruses from infected plants (Rosell *et al.*, 1999).

Whitefly colonies were housed in separate rooms to permit rearing in isolation under an identical temperature (28°C, 12 h/12 h day/night). For all transmission experiments, only adults were utilized and studies were carried out in environmentally controlled growth chambers that were isolated from all three whitefly colonies.

Chino del tomate virus *and source plants*

The whitefly-transmissible begomovirus, CdTV, was originally isolated from symptomatic tomato plants collected in Sinaloa, Mexico in 1982 (Brown & Nelson, 1988; Brown *et al.*, 2000*a*). The laboratory CdTV culture was initially established by allowing the AZ A biotype vector to transmit virus from field-infected tomato to tomato seedlings *Lycopersicon esculentum* cv. 'Pole Boy'. The CdTV culture was subsequently maintained by serial passage in tomato using the AZ A biotype whitefly as the virus vector since that time.

For vector transmission experiments, tomato plants used as virus source plants were biolistically-inoculated with total DNA extracts isolated from whitefly-inoculated, CdTV-infected plants. This was undertaken in order to establish tomato plants infected with approximately the same relative amount of initial virus inoculum for which symptom development and virus titre would be as uniform as possible. To accomplish this, total nucleic acid extracts were isolated from 1 *g* CdTV-infected plants 14 days post-inoculation by the method of Doyle & Doyle (1987). Redissolved pellets were treated with RNase (1 μg ml⁻¹) for 45 min at 37°C, DNA was extracted with phenol: chloroform: isoamyl alcohol (50:49:1), and the supernatant was precipitated with 1 vol. isopropanol and 0.5 vol. 7.5 M ammonium acetate overnight at -20°C. Pellets were collected by

microcentrifugation and redissolved in 500 ml of 10 mM Tris-EDTA buffer, pH 7.6 (TE). Approximately 0.5 mg viral DNA was precipitated onto microprojectiles, per manufacturer's instructions, and used for biolistic inoculation of tomato seedlings (Brown & Poulos, 1990). Tungsten particles coated with viral DNA were used to inoculate tomato seedlings (3-4-leaf stage) using a Du Pont biolistic apparatus, Model PDS-1000, calibrated at 1300 PSI for helium-powered particle acceleration. Experimental controls consisted of tomato seedlings mock inoculated with DNA extracts from healthy tomato. Inoculated and uninoculated control plants were placed in the growth chamber and observed periodically for symptom development over a 2-wk period of time. Seeds were sown and tomato seedlings were grown and maintained under an identical temperature and light regime (28°C, 12 h/12 h day/night) throughout this study. Under these conditions, wild type CdTV disease symptoms began to develop 8 days PI, and by 10 days PI all plants had developed typical, severe symptoms characteristic of those caused by the virus. On day 10 (PI), symptomatic plants were routinely used as virus source plants for transmission experiments.

Virus ingestion, transmission and persistence

Whiteflies were allowed an acquisition-access period (AAP) by providing adult whiteflies of the A and B biotypes of *B. tabaci* AAPs of 0.5, 1, 2, 24, 48, or 72 h on CdTV-infected tomato plants or mock-inoculated, virus-free control plants. Three replicates were carried out at each AAP, and a replicate was discarded if it was not possible to obtain at least 10 whiteflies per group. Whiteflies were collected after each AAP interval using a hand-held aspirator. The sex of each whitefly was determined by examination under a dissecting microscope. Half of each group of whiteflies (AZ A and B biotypes, and the nonvector) was placed at -80°C and later analysed for CdTV presence using polymerase chain reaction (PCR) to ascertain if virus was ingested. The remaining groups of females were used immediately in transmission studies.

For transmission experiments single females for the AZ A and B biotype were transferred, after each AAP, to virus-free tomato seedlings (3-4 leaf stage) and allowed a 72 h inoculation access period (IAP). Each AAP/IAP replicate consisted of a minimum of 10 inoculated plants for which at least whiteflies had survived both the AAP and IAP. Adult whiteflies were collected from plants, and eggs or early instar stages were killed by fumigation (Idris & Brown, 1998). Test plants were transferred to a growth chamber and maintained under the conditions described. Plants were observed periodically for symptom development over the course of 21 days

and the development of characteristic CdTV disease symptoms was considered evidence for a successful transmission event.

To compare virus detection frequency in males and females of B biotypes, adult whiteflies were allowed a 48 h AAP on virus-free tomato seedlings. Ten males and females were collected for the virus detection by PCR analysis in each of the three replicates.

To determine the duration for which virus could persist in the B biotype vector and the non-vector *T. vaporariorum* whiteflies that had been allowed to ingest virus, several hundred male and female whiteflies were given a 48 h AAP on CdTV-infected tomato plants. Whiteflies were then caged on virus-free cotton plants (8-10 leaf stage), a non-host of CdTV. Plants were maintained in a controlled growth chamber, as described. At 24 h intervals, 5-10 whiteflies were removed until all whiteflies had been collected from plants. Whiteflies were sexed and five females were stored at -80°C until analysed for virus presence by PCR.

Experimental controls were females of biotype B and the non-vector *T. vaporariorum* whiteflies from virus-free colonies, which had been given a 48 h AAP on healthy tomato plants prior to their confinement on the virus-free cotton plants.

Preparation of whiteflies for CdTV detection by PCR and Southern analysis

For detection in whiteflies, single male or female adult whiteflies were homogenised in 60 µl lysis buffer (5 mM Tris-HCl, pH 8.0), 0.05 mM EDTA, 0.5% Nonidet P-40, 1.0 mg ml⁻¹ Proteinase K on the virgin side of a sheet of Parafilm* (American National Can™) with the rounded end of a 0.4 ml plastic centrifuge tube. Particulates were removed by centrifugation at 9000 × *g* for 30 s and supernatants were used immediately for PCR. At least three replicates of 10 female whiteflies for each IAP/AAP experiment were analysed by PCR. Female whiteflies were assayed for virus presence at all time points while males were assayed following the 48 h AAP only.

Extraction of DNA from plants for PCR analysis was as described for the preparation of inocula used for biolistic inoculation of plants, except that final pellets were dissolved in TE buffer (1 g ml⁻¹).

PCR detection and Southern analysis of virus

Detection of CdTV DNA by PCR in whiteflies and plant extracts was accomplished using PCR primers designed to amplify the 'core region' of the CP gene of begomoviruses (core *CP*), yielding a diagnostic fragment 576 bp in size from CdTV infected plants (Brown *et al.*, 2000a; Wyatt & Brown, 1996). PCR primers were synthesised at the Biotechnology Facility at the University of Arizona, Tucson, AZ. PCR reaction mixtures of 25 µl were

performed in a Perkin Elmer DNA Thermal Cycler (Norwalk, CT) under conditions described previously (Rosell *et al.*, 1999). Negative controls included the PCR reaction mixture without DNA template, or with 1.5 µl of non-viruliferous whitefly homogenate. Additional negative and positive controls were template total nucleic acids extracted from virus-free tomato leaves, and total DNA extracted from the virus source tomato plants infected with CdTV, respectively.

PCR products were electrophoresed in 1% agarose gels in TAE. Gels were stained with ethidium bromide and PCR products were viewed by UV transillumination. The relative CdTV DNA concentration in representative virus source plants and whiteflies was estimated by the intensity of ethidium bromide staining of core CP fragments, compared to a dilution series of two internal standards (100 bp ladder (GIBCO, BRL) and lambda digested with Hind III (data not shown).

To confirm that PCR products were of begomoviral origin, amplicons resolved by agarose gels were transferred to nylon membranes by capillary transfer and subjected to Southern blot hybridisation as described (Rosell *et al.*, 1999). The DNA probe consisted of a cloned A component of CdTV containing the full viral CP gene (Brown *et al.*, 2000*a*), and hence was expected to hybridise to the core CP region nested within the CP ORF, and amplified from whiteflies and plants. The DNA probe was labeled by random priming with digoxigenin and hybridization and immunodetection were as described (Rosell *et al.*, 1999).

Statistical analysis

Statistical analyses were conducted using the general linear model (GLM) and Spearman's rank correlation (CORR) procedures of the Statistical Analysis System version 6.11 (SAS Institute Inc., Cary, NC). Each experiment was evaluated in a split-plot design, with vectors assigned to main plots and CdTV to a subplot. Correlations were calculated between ingestion, and the frequency of virus detection, and frequency of transmission, as determined by bioassay to tomato, for each individual whitefly. To analyse prospective interactions between the two whitefly vectors and CdTV ingestion and transmission frequencies, and between CdTV and the greenhouse whitefly (nonvector), pairwise comparisons of the means were made using the *t*-test generated by the PDIFF option of GLM. Significant differences were calculated at $P < 0.05$.

Results

Virus-vector experiments

Neither vector whitefly transmitted CdTV given an AAP of 0.5 h or less. The AZ A biotype vector transmitted CdTV with a frequency of 3%+ following a 1 or 2 h AAP, and as AAPs were increased, transmission frequencies increased to 30% following a 24 h AAP and 50% transmission for a 72 h AAP (Fig. 1*a*). In contrast, the B biotype did not transmit CdTV following AAPs of less than 2 h (Fig. 1*a*), and did so only following a 24 h AAP, for which transmission frequency was 27%. The B biotype was thereafter able to transmit CdTV for AAPs greater than 24 h (Fig. 1*a*), however despite increasingly longer AAPs (48 and 72 h), transmission frequencies did not increase beyond that attained with a 24 h AAP (Fig. 1*a*). Interestingly, when both biotype vectors were allowed the maximum (72 h) AAP, CdTV was transmitted nearly twice as often by the New World AZ A biotype (50%) (Fig. 1*a*), compared to 27% by the Old World B biotype vector. As expected, neither vector transmitted CdTV when given a 24 AAP on virus-free tomato control (Fig. 1*a*).

Statistical analysis of the mean for CdTV transmission indicated that single AZ A biotype females were significantly more efficient vectors of CdTV, a New World tomato-infecting virus, than were females of the Old World B biotype vector (Fig. 1*a,b*).

Virus ingestion determined by PCR and Southern analysis

CdTV DNA was detectable in single A and B female biotype whiteflies given AAPs on CdTV-infected tomato plants ranging from 0.5 h to 72 h (Fig. 1*b*), while, as expected, CdTV was also detectable in single non-vector (control) whiteflies (data not shown) that were allowed identical AAPs on virus-infected plants.

PCR detection results indicated that CdTV was ingested at a frequency of 3% for single A and 13% of single B biotype whiteflies, given a brief (0.5 h) AAP, indicating that both vectors had ingested virus, given only minimal exposure to the virus source, even though they were not yet able to transmit virus. For both the A and B biotype vectors, the frequency of virus detection increased as whiteflies were allowed longer AAPs, ultimately, reaching 100% and 97% for A and B biotypes, respectively, following a 72 h AAP (Fig. 1*b*). Overall, however, virus DNA was detectable by PCR in more B biotype than in AZ A biotype individuals throughout the experiment, irrespective of the specific AAP. Yet, the AZ A biotype transmitted the virus with significantly shorter AAPs, and more frequently following all AAPs tested here (Fig. 1*b*).

Statistical analysis of mean percentage PCR detection of CdTV indicated that virus was detectable in a significantly greater number of B biotype females, compared to the AZ A biotype vector given 0.5-2 h AAPs, prior to completion of
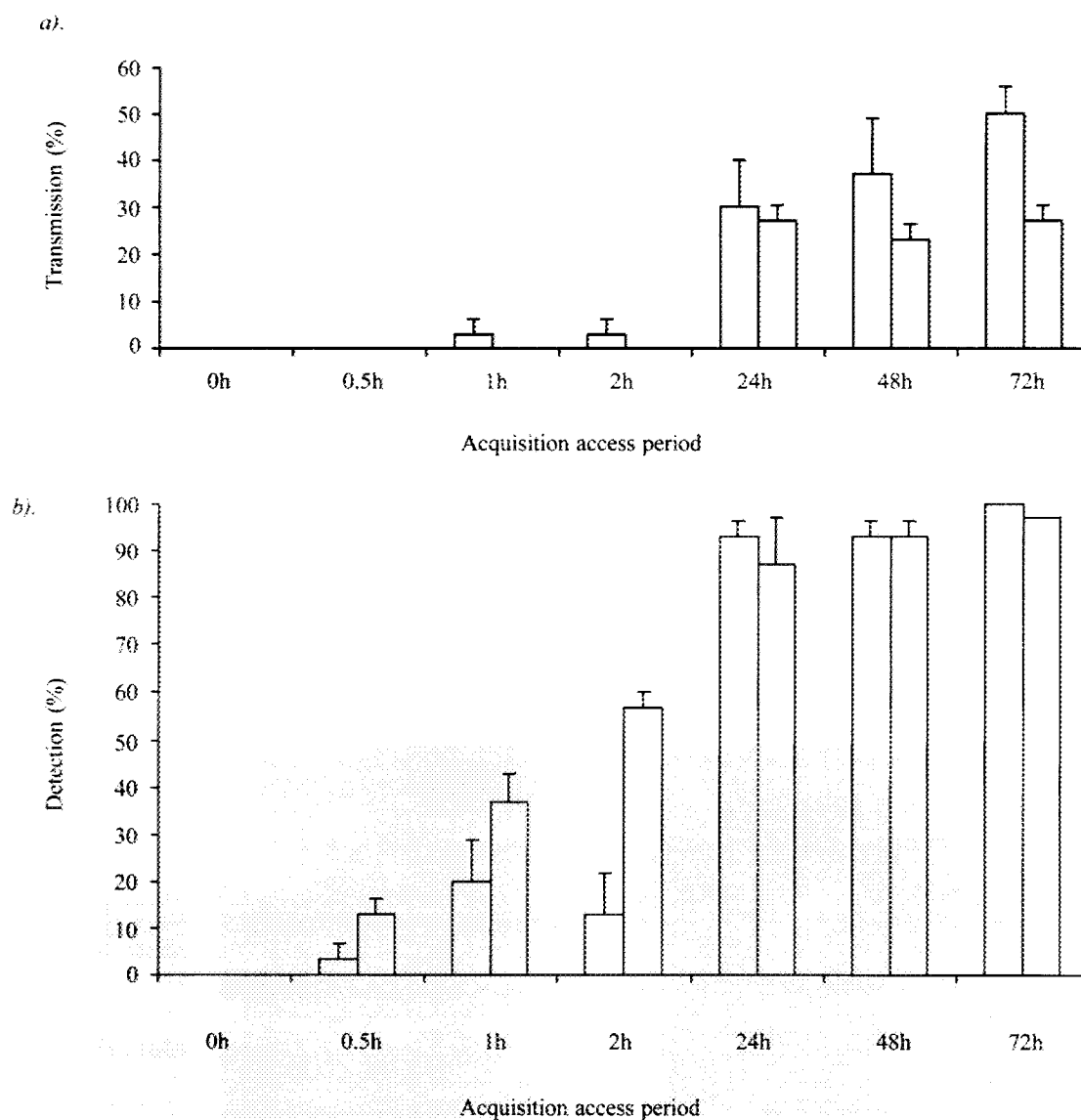
a).

b).

Fig. 1. Single whitefly transmission of *Chino del tomate virus* (CdTV) from Mexico by two vector biotypes and begomovirus detection by polymerase chain reaction in single adult whiteflies. *a)*. Acquisition-access periods in whitefly transmission assays ranged from 0-72 h with a 72 h inoculation-access period. The AZ A and AZ B biotype vectors were used to transmit CdTV. Percent transmission is shown as the mean of three replicates, using ten test plants per replicate. *b)*. Polymerase chain reaction detection of CdTV in single whiteflies after acquisition access periods ranging from 0 to 72 h. Percent detection is presented as the mean of three replicates, with ten whiteflies each. Vertical bars indicate the standard error. Shaded bars, A biotype; unshaded bars, B biotype.

the latent period. However, CdTV was equally detectable in females of both vector biotypes following 24 h, 48 h and 72 h AAP (Fig 1*b*), all experiments for which the latent period had been satisfied (Brown & Nelson, 1988).

A subsequent comparison of the frequency with which CdTV could be detected by PCR in males and females of the least competent vector, the B biotype, revealed that 60% and 96% of males and females contained detectable virus, respectively, following an AAP of 48 h for which the latent period had already been satisfied. Statistical analysis

indicated that significantly fewer males than females contained detectable CdTV (Fig. 2).

Southern hybridisation results confirmed the identity of the 576 bp amplicon as CdTV DNA that was detectable in both the Old and New World vector biotypes, and in the non-vector whitefly that was capable of ingesting virus from infected plants. No PCR product of the expected size was detected in control female whiteflies allowed a 48 h AAP on virus-free, healthy control tomato plants. Likewise, the CdTV probe hybridised to PCR amplicons obtained from extracts of CdTV-infected, but
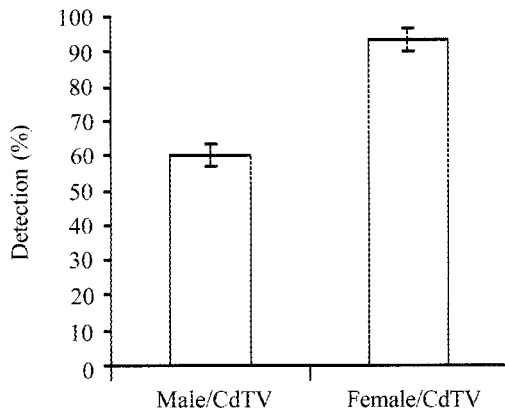
Fig. 2. Detection frequency by polymerase chain reaction assay of *Chino del tomate virus* in single AZ B biotype males and females. Whiteflies were allowed a 48 h acquisition access period on virus-infected tomato plants. Percent detection is shown as the mean of ten single whiteflies in each of the three replicates. Vertical bars indicate the standard error.

hybridisation was not observed in lanes containing PCR reactions with extracts of virus-free control plants added as template (Fig. 3).

Persistence of CdTV in vector and non-vector whiteflies

CdTV was detectable by PCR for at least nine days after female B biotype whiteflies were given an AAP of 48 h on virus from infected plants, even though whiteflies were maintained on cotton, a non-host of the virus after the initial exposure to virus. Based on PCR, the frequency (95-100%) with which detectable virus persisted in single whiteflies was relatively constant from day 1-9, except on day 4, when the detection frequency declined to 90%.

In contrast, CdTV was detectable by PCR in single female nonvector whiteflies (*T. vaporariorum*), which had been given a 48 h AAP for only 5 days after initial virus exposure. In the non-vector, CdTV detection decreased rapidly from a high of 100% on day one, to 40-50% on the second
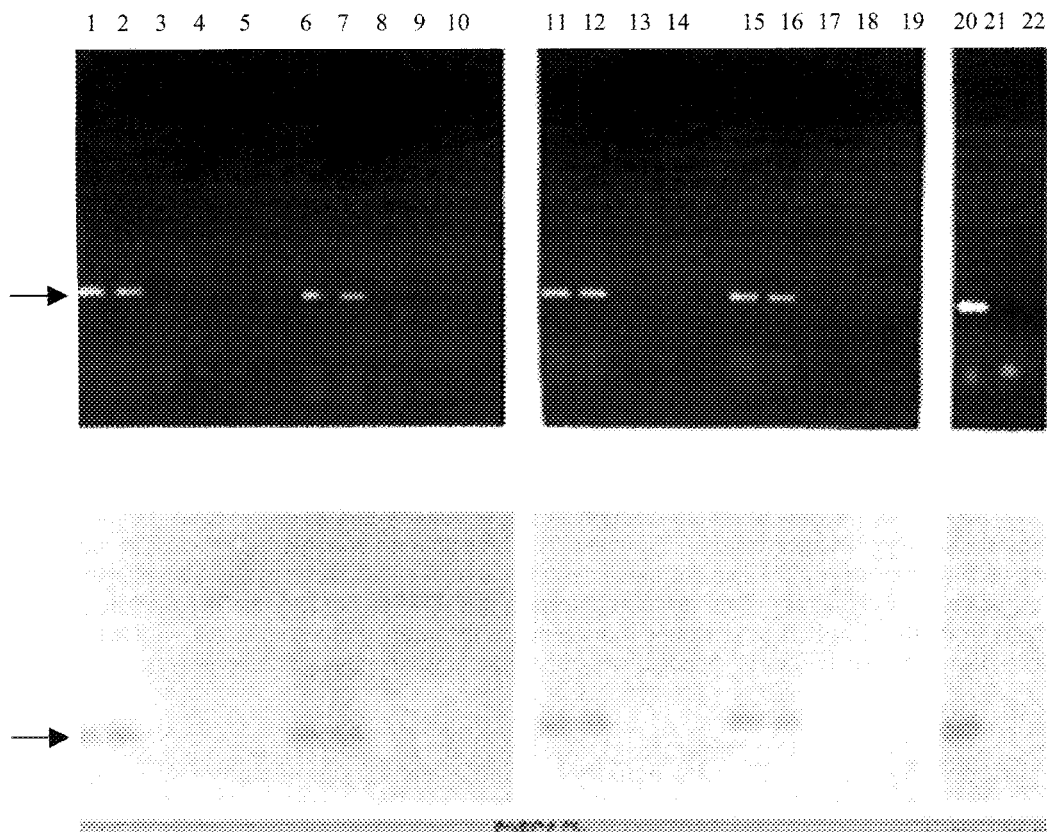

Fig. 3. Top panel: An ethidium bromide-stained agarose gel (1%) with separated PCR products for extracts of individual whiteflies. Whiteflies were given a 48 h AAP on *Chino del tomate virus* (CdTV)-infected or on healthy tomato plants. Lanes 1 and 2 contain female AZ A biotype (*B. tabaci*), lanes 6 and 7 contain female greenhouse whitefly (*T. vaporariorum*), lanes 11 and 12 contain female AZ B biotype (*B. tabaci*) and lanes 15 and 16 contain male AZ B biotype fed on virus-infected plant; lanes 3 and 4 female AZ A biotype (*B. tabaci*), 8 and 9 female greenhouse (*T. vaporariorum*), lanes 13 and 14 female AZ B biotype (*B. tabaci*) and lanes 17 and 18 male AZ B biotype (*B. tabaci*) fed on healthy tomato plants; lanes 5, 10, 19, and 22 contain PCR reaction mixture with no DNA template; and lanes 20 and 21 are PCR products from CdTV-infected and healthy control plants, respectively. Arrow indicates the 576 bp marker. Bottom panel: Southern blot of gel in top panel probed with digoxigenin-labeled CdTV A component.
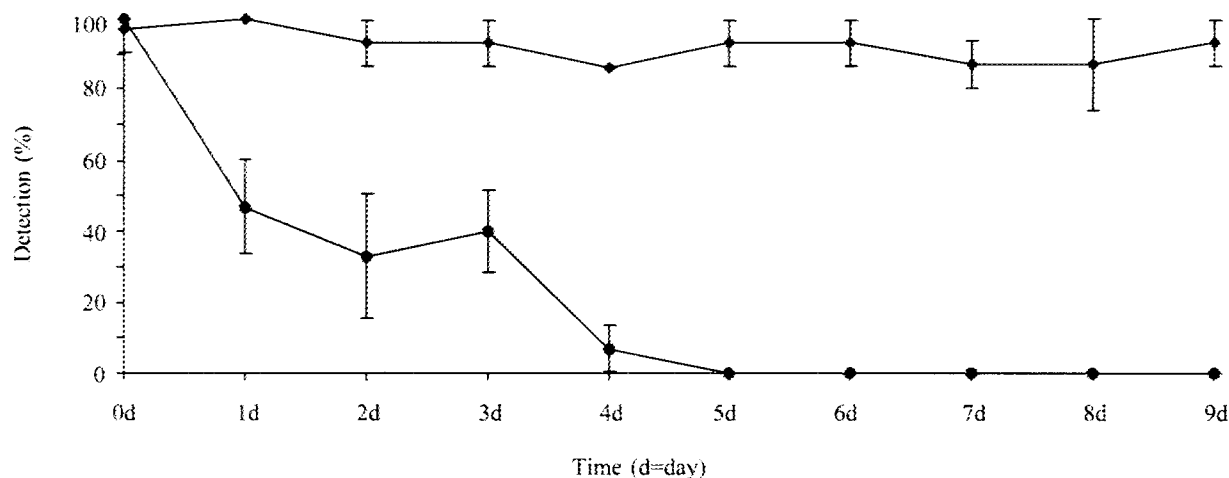
Fig. 4. Persistence of *Chino del tomate virus* (CdTV) in single vector (*B. tabaci* AZ B biotype) and non-vector (*T. vaporariorum*) whiteflies. Whiteflies were provided a 48 h acquisition access period on virus-infected tomato plants and transferred to cotton plants (a non-host of CdTV). Female whiteflies were collected from cotton plant for virus detection at 24 h intervals for nine consecutive days using polymerase chain reaction. Day zero indicates that detection assay was performed immediately after a 48 h acquisition access period. Percentage detection is shown as the mean of three replicates with ten whiteflies each. Vertical bars indicate the standard error.

and third days and to less than 10% by day 4. On day 5, virus could not be detected in the nonvector whitefly (Fig. 4).

Discussion

In its natural habitat, CdTV (Brown & Nelson, 1988) has long been transmitted by a A-like biotype of *B. tabaci* (Costa & Brown, 1991; Brown *et al.*, 1995a; Frohlich *et al.*, 1999), and only recently by the B biotype which was introduced into the region in 1987-88 (Costa & Brown, 1991; Costa *et al.*, 1993). New World A-like biotypes of *B. tabaci* and the respective begomoviruses they transmit have apparently co-evolved in isolation (Brown, 2001a) and, therefore, might be expected to have developed a relationship owing to these interactions that is manifest, in part, as selective transmission. In this study, we observed a positive correlation between PCR detection of virus in whitefly vector variants from either the Old and New World, respectively, and frequency of whitefly-mediated transmission of CdTV, a New World virus. Although CdTV DNA was detectable by PCR in the whitefly, *T. vaporariorum*, that shares a similar host range and behavioral characteristics in common with *B. tabaci*, CdTV was not transmissible by this whitefly.

There is growing evidence that whitefly vector-begomovirus combinations have co-evolved primarily with geographic and, more rarely, with host constraints. Evidence suggests that transmission frequency may be enhanced for such complexes, compared to that for geographically isolated virus-vector combinations. However, the observed enhancement is often subtle and the mechanisms involved in selective transmission are not known.

Members of the *B. tabaci complex* presently are known to transmit most begomoviruses, irrespective of geographical origin of virus or vector, providing vector-host incompatibility does not preclude the opportunity (Bedford *et al.*, 1994; Bird, 1957; Burban *et al.*, 1992; Brown & Bird, 1996; Idris, 1997; McGrath & Harrison, 1995). Although speculative, co-evolution of selective transmission may be a relatively recent phenomenon that may have occurred concomitantly with the putative speciation of the *B. tabaci* complex, thought to be underway (Brown, 2001a; Frohlich *et al.*, 1999). Further isolation might be expected to increase virus-vector specialisation and virus-vector interdependence that could ultimately lead to development of mechanisms for replication and transovarial passage in their whitefly vector for certain viruses, as has been reported for *Tomato yellow leaf curl virus*-IL (Morin *et al.*, 1999).

Although differences in vector competency for a particular virus, in relation to co-geographical isolation (or exclusion) within the Americas and Caribbean region, is poorly studied, results indicate that the B biotype is not a highly competent vector of the New World CdTV, compared to the A biotype, which shares a common geographical origin with CdTV. CdTV was detectable by PCR in both Old and New World vectors allowed only a brief (0.5 h) AAP on infected plants, but only the A biotype was able to transmit CdTV under the respective assay parameters. In contrast, the B biotype transmitted CdTV only when allowed a substantial AAP of 24 h or more, even though ingestion frequencies by both vectors were nearly identical. These results suggest that CdTV is transmitted with greater competency, and possibly 'selectivity', by its New World A type

vector, than by the Old World B biotype. Finally, because the B biotype was previously host-adapted to, and more fecund on, tomato than the A biotype (authors, personal observation), host incompatibility does not seem a likely explanation for the observed discrepancies in acquisition and transmission frequencies between the two vectors. These observations, collectively, suggest that the *B. tabaci*-begomovirus transmission pathway may harbor subtle discriminating or selective barriers that are most compatible between co-evolved virus-vector combinations, and that selectivity operates either before and/or during the latent period, implicating the gut and/or salivary gland membranes as critical barriers.

Whitefly-mediated transmission of begomoviruses is thought to involve recognition between the viral capsid and whitefly vector receptor-like molecules that facilitate viral transport across the gut membrane and entry into the accessory salivary glands (Briddon et al., 1990; Cicero *et al.*, 1995; Ghanim *et al.*, 2001; Hunter *et al.*, 1998; Rosell *et al.*, 1999). In the haemolymph, virions are probably 'protected' by GroEL, a whitefly-associated chaperonin of prokaryotic origin (Morin *et al.*, 1999). In the transmission pathway, encapsulated and 'GroEL-protected' virions are probably inaccessible to detection methods commonly employed, making it difficult to assess virus titre and track virus in the pathway (Cohen *et al.*, 1989; Rosell *et al.*, 1999; this report). Collectively, studies have shown that detectable virus increases steadily following an optimal AAP and declines 17-26 h later (Brown & Nelson, 1988; Cohen *et al.*, 1989; Rosell *et al.*, 1999). The decline in detectable virus roughly corresponds to the culmination of the latent period, which is about 8-12 h for CdTV (Brown & Nelson, 1988), and first evidence of transmission, which requires virus acquisition in the interim. In non-vector whiteflies, virus levels have been shown to increase rapidly to high levels followed by a rapid decline with minimal or no detection several days later (Rosell *et al.*, 1999; this report). In this study, CdTV was detectable in the less-competent B type vector sooner and at higher frequencies than with the adapted A biotype, yet CdTV transmission frequencies by the A type vector were twice as high as for the B type. Collectively, these data suggest that a more competent vector may sequester a co-evolved virus sooner and release it more slowly than a less-competent vector, and that the inability to detect virus in the vector may be indicative of binding, encapsulation, and/or acquisition involving the various membrane-bound, receptor-like and other soluble factors that may bind virus during the transmission pathway. In this light, it is difficult to reconcile results from two other studies in which greater frequencies of virus detection than transmission were reported for co-evolved virus-vector combinations (Mehta *et al.*, 1994; Polston *et al.*, 1990).

CdTV was detected in only about half the B biotype males (60%), compared to females (100%), despite a 48 h AAP and sufficient latent period (Fig. 2), suggesting that a typical population containing both males and females would be even less competent vectors of CdTV, than females alone. In general, females have been reported as more efficient vectors than their male counterparts (Cohen & Nitzany, 1966; Nateshan *et al.*, 1996). CdTV persisted in its whitefly vector for at least 9 days (or longer) after a 48 h AAP, which is in agreement with transmission studies for CdTV in which the A type vector was used (Brown & Nelson, 1988). Apparently, this New World virus persists in both adapted and non-adapted *B. tabaci*, thereby potentially securing the vector for the duration of its life, and suggesting that selectivity may operate primarily during ingestion and acquisition stages and that it is less important during the transmission stage.

Given that a single whitefly is rarely associated with an epidemic, 50% transmission competency and a relatively brief AAP suggests that very few viruliferous A biotype whiteflies are needed per plant to reach 100% disease incidence, all things being equal. In contrast, transmission of CdTV by the B biotype required a 24 h exposure to the virus source, suggesting that non-adapted virus-vector combinations may exhibit different patterns of transmission and spread than co-adapted combinations. Despite less-than-optimal transmission by the B biotype of several other New World viruses (Bedford *et al.*, 1994; Brown & Nelson, 1989; Brown *et al.*, 1999), its ability to reach unprecedented population densities (Bedford *et al.*, 1994; Costa & Brown, 1991) and displace many local populations, together with the capacity to acquire and transmit New World begomoviruses, may explain the increased frequency with which begomoviruses emerged in the Americas and Caribbean region (Brown, 1994; Brown & Bird, 1992). Although a longer AAP is required for CdTV transmission and it is transmitted only half as efficiently by the Old World vector, the substantial reproductive capacity of the B biotype may more than compensate for its lowered vector competency. Nonetheless, these results suggest that selectivity occurs between certain co-adapted virus-vector combinations, and that it probably occurs prior to the latent period and/ or during acquisition, and possibly during the early transmission stage, rather than during ingestion or late transmission stages. However, the rapidity with which begomoviruses have emerged as pathogens in the New World following invasion by the B biotype vector (Brown, 2001*a,b*; Costa & Brown, 1991; Costa *et al.*, 1993; Frohlich *et al.*, 1999) underscores the importance of knowledge regarding distinguishing biological characteristics of the vector, including fecundity, host adaptability, among others, that can

feasibly influence the emergence or the extinction of begomoviral pathogens, even when selective interactions may be operational at higher levels.

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