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Post-release evaluation of biological control of *Bemisia tabaci* biotype “B” in the USA and the development of predictive tools to guide introductions for other countries

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Abstract

Climatic matching and pre-release performance evaluation were useful predictors of parasitoid establishment in a retrospective analysis of the classical biological control program against *Bemisia tabaci* biotype “B” in the USA. Laboratory evaluation of 19 imported and 2 indigenous parasitoid species in quarantine on *B. tabaci* showed that the Old World *Eretmocerus* spp. had the highest attack rate. The climate matching program CLIMEX was used to analyze the establishment patterns of five Old World *Eretmocerus* spp. introduced to the Western USA. The top matches $\pm 10\%$ for the climate of the area of introduction and origin of the introduced parasitoids always included the species that established. The Old World *Eretmocerus* spp. came from regions characterized by many separate biotypes of *B. tabaci* other than “B”, but are considered specialists of the *B. tabaci* complex as compared to the indigenous North American oligophagous *Eretmocerus* spp. This narrower host range and high attack rate combined with climatic adaptation may account for their establishment in the USA. A set of predictive tools and guidelines were used to select the best candidate for importation and possible release into Australia that has been recently invaded by the “B” biotype. The establishment patterns of the introduced *Eretmocerus* spp. and a comparison of climates of their respective locations in the USA were compared with the affected area in Australia. The best climatic match was the Lower Rio Grande Valley of Texas suggesting its dominant parasitoid, *E. hayati* ex. Pakistan be considered as the first candidate for evaluation as a biological control agent.

Key words: climate matching, predictive evaluation, *Eretmocerus*, *Encarsia*, silverleaf whitefly, USA, Australia

1. Introduction

The practice of modern classical biological control has a long and distinguished history and is often the only long-term sustainable solution to problems caused by exotic pests. One of the criticisms of classical biological control is its lack of predictability in terms of agent establishment and success. Many authors have proposed that biological control needs to make the transition from an empirical method to a predictive science (Greathead, 1986; Ehler, 1990, Hoddle & Syrett 2002). While predictive tools such as pre-release evaluation of prospective agent efficacy (Waage, 1990; Ehler, 1990; Roush, 1990), climate matching between the geographic area of the pest and prospective control agents (DeBach 1964, Roush 1990, Sutherst *et al.*, 1999; Cameron *et al.*, 1993) and assessment of the agent intraspecific variation (Roush, 1990; Goldson *et al.*, 1997) have been promoted, rarely has their combined value been tested as part of classical biological control programs.

The biological control program for the silverleaf whitefly (SLW) *Bemisia tabaci* biotype 'B' Gennadius (= *Bemisia argentifolia* Bellows and Perring) provides the opportunity to test retrospectively in a preliminary manner, the validity of these predictive tools. In the SLW biological control program, a combination of climate matching, laboratory and field cage performance studies, genetic assessment and subsequent field establishment were used to guide the search, evaluation and prioritization for release of exotic parasitoids into the Western USA. Information on long-term establishment of agents is now available from releases made in Texas (TX), Arizona (AZ) and California (CA) during the mid 1990's. This paper is the first to report on long term establishment in the USA of the *Eretmocerus* spp. introduced for biological control of *B. tabaci*.

Bemisia tabaci is known to be a potentially damaging pest of crops such as cotton, brassicas, cucurbits, okra, solanums in the tropics and subtropics. Damage is caused not only by direct feeding, but also through transmission of geminiviruses (Brown, 1994; De Barro, 1995; Alegbejo, 2000; Morales & Anderson, 2001). In response to a series of outbreaks of the B biotype in the southern USA during the 1990's, an international search for parasitoids of SLW was implemented and as a result over 56 populations of parasitoids were established in quarantine culture from collections made between 1992 and 1998 (Kirk *et al.*, 1993; Kirk & Lacey, 1995; Legaspi *et al.*, 1996; Kirk *et al.*, 2000). The exploration effort utilized climatic matching to prioritize areas for surveys. Imported natural enemies were evaluated in laboratory and field tests and then released in AZ, CA, and TX (Goolsby *et al.*, 1996; Goolsby *et al.*, 1998; Gould *et al.*, 1998; Hoelmer *et al.*, 1998; Simmons *et al.*, 1998; Pickett *et al.*, 1999).

We now evaluate the outcomes of this approach in order to develop a set of tools with which to assess parasitoids of *B. tabaci* being considered for importation by other countries. To put these tools into practice we then use them to predict which parasitoid(s) would be the best candidate(s) for introduction into those regions of Australia currently affected by SLW. Such an approach is of particular use to countries where the high costs associated with biological control programs potentially limit the number of taxa that can be considered for introduction.

2. Materials & Methods

To test the hypothesis that pre-release quarantine evaluation and climatic matching were useful predictors of parasitoid establishment we used data from post-release surveys conducted from 2001 to 2003. Pre-release quarantine evaluation trials were reviewed to determine which parasitoids performed best (Goolsby *et al.*, 1996; Goolsby *et al.*, 1998). Results from an evaluation trial of parasitoids attacking *B. tabaci* infesting broccoli is presented in the results. Although considerable information was available from field evaluation that confirmed results from quarantine screening, we opted to use only the latter in our post-release evaluation. We did this to simulate the actual pre-release decision making process of a country faced with multiple choices for importation of natural enemies and to develop guidelines for future biological control programs.

Determinations of parasitoids recovered from post-release surveys were made using both morphological and molecular methods. Morphological determinations were based on characteristics of slide mounted adult females using a phase contrast compound microscope. The following species (name plus Mission quarantine facility accession #) were identified using characters in the key by Zolnerowich and Rose (1998): *Eretmocerus mundus* Mercet (M92014), *Eretmocerus melanoscutus* Zolnerowich and Rose (M94036) *Eretmocerus hayati* Zolnerowich and Rose (M95012), *Eretmocerus emiratus* Zolnerowich and Rose (M95104). *Eretmocerus* sp. (M96076 - Ethiopia) was identified by comparison to the original parental material imported into quarantine in 1996. For molecular identifications we sequenced the ribosomal ITS1, which ranged from 549 to 555 base pairs long depending on the species (De Barro *et al.* 2000a). Only parasitoids recovered after 2001 were included in the study. This date is two and four years after the last release of the imported *Eretmocerus* spp. in CA-AZ and TX respectively.

Climates at the centers of origin of the top performing parasitoids were compared with those in the Western USA (AZ, CA and TX) where releases were made. The Eastern USA was excluded due to the lack of information on releases and recoveries of introduced parasitoids. We used the United Nations, Food and Agriculture Organization website for Sustainable Development (<http://www.fao.org/ag/agl/agll/gaez/index.htm>) to provide general descriptions

of climate to broadly distinguish the regions in this study. The ‘*Match Climates*’ function in the CLIMEX software (Sutherst *et al.* 1999) was used to compare the climatic averages of original parasitoid collection sites with those of the release sites. The level of similarity is given by the ‘Match Index’, which is the average of up to six component indices which indicate the degree of similarity of the maximum, minimum and average temperatures, total rainfall, rainfall pattern, relative humidity and soil moisture. Each of these component indices can range between 0 and 100, with a value of 100 indicating an exact match of the two locations. In our study, comparisons of average temperature, humidity, and total rainfall and rainfall pattern were excluded from the analysis. We have found that soil moisture was a better indicator of ambient RH. Ambient RH may be significantly different from field sites where it can be influenced by irrigation and transpiration rates of the crop plants. Since both the host insect and parasitoid have multiple overlapping generations and no diapause, we excluded the rainfall and rainfall pattern index, which we assumed, would not have a significant impact on their phenology. If locations from different hemispheres are compared, CLIMEX automatically displaces the data from the Southern Hemisphere location by six months. The version of CLIMEX used (‘*CLIMEX for Windows Patch 1*’ on the website of the publisher) was that modified to correct earlier errors in the Match Climates algorithm. We considered the best climatic matches from the Old World and Western USA locations to be the top match index plus those within 10%. Climatic data for, San Joaquin Valley, Lower Rio Grande Valley, Imperial Valley and Yuma came from weather stations reporting from; Bakersfield, CA; McAllen, TX; Brawley, CA and Yuma, AZ, respectively. Climatically, Brawley and Yuma are essentially the same (Match Index = 95) and are treated as the same climate in the discussion section. For climates in the origins of the *Eretmocerus* spp. the United Arab Emirates (UAE), Ethiopia, Pakistan, Thailand and Spain we used climate data from Sharjah, Awash, Multan, Chiang Mai and Murcia, respectively.

For the selection of agents for release in Australia we matched climates in the Western USA where parasitoids had been released with the SLW affected areas of subtropical and tropical eastern Australia using CLIMEX indices. We then used establishment data from the matching site in the USA to determine the best candidate parasitoid(s) for Australia.

3. Results & Discussion

3.1 Pre-release quarantine evaluation

The pre-release quarantine evaluation has been previously published and readers are referred to Goolsby *et al.* (1998) for methodology. In brief, the lifetime fecundities of the parasitoid females representing several species in quarantine culture were evaluated in sleeve cages on three separate hosts, cotton, melons, and broccoli infested with

B. tabaci immatures. Despite the space limitations imposed by rearing facilities generally available in quarantine facilities, simple pre-release evaluation using standard rearing conditions provided an insight into the potential of the various agents under consideration. Several of the species, which eventually established showed high attack rates in this evaluation. These assessments also suggested that the *Encarsia* spp. (Hymenoptera: Aphelinidae) generally showed less potential than *Eretmocerus* spp. (Hymenoptera: Aphelinidae) (Goolsby *et al.*, 1998) (Table 1). The testing ranked two of these species the highest (*E. mundus* and *E. hayati*), which eventually became established in the field. Predictive testing did not show the potential of *E. emiratus* or *Eretmocerus* sp. (Ethiopia), as both were obtained after the bulk of the pre-release assessments had been completed. Never the less, the parasitoids that did best in two of the three climates where releases took place, were those that had the highest attack rates. While attack rate may not always be the most important factor in relation to establishment or success of the parasitoid species, it was a good indicator of eventual establishment in our study. Why this is the case is open to speculation, but may be related to the ephemeral nature of the cropping systems affected. One *Encarsia* species, *E. sophia*, which demonstrated a low attack rate in the pre-release studies, did eventually establish in the Imperial Valley. However the bulk of the parasitoids recovered in the Imperial Valley from 2000 onwards are the introduced *Eretmocerus* spp. (Roltsch, unpublished data).

3.2 Post release evaluations

Small scale releases into field cages and free releases into long-term garden plots generally confirmed the rankings indicated by the pre-release studies (Hoelmer, 1998; Goolsby *et al.*, 2000; Pickett *et al.*, 2001; Roltsch, 2001, Hoelmer & Goolsby, 2002). Following widescale release, post-release surveys over the western USA confirmed the above evaluations (Table 2). As mentioned above, of the seven *Encarsia* spp. released only one, *E. sophia* (Viggiani) established (Roltsch, 2001). In contrast, of the five species of Old World *Eretmocerus* spp. released, four species *Eretmocerus mundus* (India, Mediterranean Basin), *E. hayati* (Pakistan, north western India), *E. emiratus* (United Arab Emirates) and *Eretmocerus* sp. (Ethiopia) established. A fifth species, *E. melanoscutus* (Thailand, Taiwan) failed to establish. Based on the above we now focus on *Eretmocerus*.

3.3 Intraspecific variation within *Eretmocerus* and *Bemisia tabaci*

The four species of Old World *Eretmocerus* that established in the Western USA are morphologically similar and represent one genetic group of very closely related taxa that appear to be specialist parasitoids of the *B. tabaci* complex (De Barro *et al.*, 2000a; Zolnerowich & Rose, 1998). The specialization of these species on the *B. tabaci* complex may have given them an advantage in the field versus the native *Eretmocerus tejanus* Rose & Zolnerowich

and *Eretmocerus eremicus* Rose & Zolnerowich which are known to have a broader host range including *Trialeurodes* spp. (Rose and Zolnerowich, 1997; Hoelmer & Goolsby, 2002).

Further, *B. tabaci* is composed of a number of genetically related populations that occupy distinct, separate geographic distributions (Frohlich *et al.*, 1999; De Barro *et al.*, 2000b). Of particular interest is the observation that none of the Old World *Eretmocerus* which established, came from the genetic group to which the B biotype belongs. The genetic differences between the clades identified by Frohlich *et al.* (1999) and De Barro *et al.* (2000b and submitted) suggests that the populations from which the parasitoids were obtained have been separated for tens of thousands of years. This suggests that the interaction between these Old World *Eretmocerus* and the B biotype is either a new association, as proposed by Hokkanen and Pimentel (1989) as a means of improving the prospects for successful biological control, or that all *B. tabaci* genotypes are essentially equally acceptable and that some other factor such as climate is the overarching determinant of efficacy.

3.4 Climate matching

The climate in the zone of origin of each of the *Eretmocerus* spp. released was different, *E. mundus* (Mediterranean), *E. emiratus* (hot desert), *Eret. sp.* (Ethiopia) (tropical desert), *E. hayati* (subtropical desert), *E. melanoscutus* (tropical monsoon). The climates where the species were introduced can be described as follows, San Joaquin Valley (Mediterranean), Imperial Valley and Yuma (hot desert), Lower Rio Grande Valley of Texas (subtropical desert). Based on the simple comparison of average monthly maximum and minimum temperatures, and soil moisture (an indicator of RH), the species which eventually dominated collections in the southwestern USA were those with a CLIMEX Match Index number that fell within 10% of the maximum rank for a particular region (Table 1).

CLIMEX comparisons are based on long-term averages and do not account for infrequent high or low temperatures. Geography influences these extremes of climate. We believe that the geography of Eurasia and North America may also help further explain the establishment patterns of the introduced *Eretmocerus* spp. in the Western USA. One of the hallmarks of the North American climate, especially east of the Rocky Mountains, is the dramatic shift in temperatures caused by polar air masses. North to south mountain ranges funnel cold air south accentuating the effect of the cold front, occasionally producing deep freezes even as far south as the Lower Rio Grande Valley of Texas. This effect called the 'climatic trumpet' (Flannery 2001) is less pronounced in the Western US because of the buffering effect of the Rocky Mountains. Therefore, *Eretmocerus* spp. introduced into Texas need to be tolerant of cold, sometimes freezing weather. Parasitoids originating from Sharjah, UAE and Awash, Ethiopia where absolute

minimums recorded are 5 and 10°C respectively and sharp drops in temperatures are uncommon, may not be able to tolerate the rapid temperature fluctuations and freezing temperatures experienced in TX. This could explain why *E. emiratus* and *Eretmocerus* sp. (Ethiopia) failed to establish in TX, but established in the more buffered climates of the Imperial Valley and Yuma. In contrast, *E. hayati* from northern Pakistan at 30 ° N, which experiences winter minimum temperatures near 3°C and the occasional freeze would be predicted to have some cold tolerance and this may explain its establishment in the Rio Grande Valley. *Eretmocerus* spp. introduced into the hot arid climates of the Imperial Valley and Yuma need to have extreme heat tolerance. *Eretmocerus emiratus* and *Eretmocerus* sp. from the United Arab Emirates and Ethiopia respectively, both come from hot arid climates with occasional temperatures near 50 °C and it is not surprising that they have the capacity to cope with the high temperature extremes of the Imperial Valley and Yuma. However, this does not explain why *E. hayati* failed to establish in either the Imperial Valley and Yuma. We have proposed that *E. hayati* has good cold tolerance, but one would expect a high degree of heat tolerance as it comes from the Indus River valley where summer temperatures reach the mid 40's. This suggests some other factors may be operating in determining establishment, but what they are remains unknown. In contrast to the three *Eretmocerus* species from desert climates, *Eretmocerus melanoscutus*, from tropical Southeast Asia where temperature extremes are rare, most likely lacked both the heat and cold tolerance necessary for establishment in any of the locations in the Western USA. However, it should be noted that this species might be an ideal candidate for release in Florida (CLIMEX match index for Ft. Meyers, FL = 73).

3.5 Summary of climate and pre-release evaluations – a stepwise process for agent selection

For each region, climate matching predicted the establishment of parasitoid species if their match index fell within 10% of the maximum value. Subsequent pre-release evaluations in the laboratory and field confirmed that generally, *Eretmocerus* spp. had the highest potential, including *E. mundus* and *E. hayati*. Post-release surveys confirmed the establishment of one or more of the top ranked species for San Joaquin Valley (*E. mundus*), Rio Grande Valley (*E. hayati*), and the Imperial Valley/Yuma (*Eretmocerus* sp. (Ethiopia)). This suggests that a stepwise process of climate matching followed by pre-release evaluations effectively identified many of the introduced parasitoid species that would eventually become established in each of the three regions within the Western USA. Although climate matching and pre-release evaluation were not perfect predictors, their usefulness for prioritizing agents is apparent.

3.6 Application of predictive indicators for selection of species for Australia

Silverleaf whitefly is now a damaging pest along the east coast of Australia extending from Bowen/Burdekin, Queensland in the north to north coastal New South Wales in the south (Fig 1). Despite early promise, species of

parasitoids already in Australia have proven to be ineffective in controlling this pest (De Barro *et al.*, 2000c, De Barro, unpublished data) and a decision to import exotic parasitoids has been made. To guide this effort, we use the above stepwise process to identify the best candidate for introduction into Australia.

Based on laboratory and subsequent field performance, the four species of *Eretmocerus* are the only taxa that should be considered for introduction. CLIMEX was then used to compare the climates of the three regions of the southwestern USA where *Eretmocerus* was released, against the regions of Australia currently affected by this pest (Fig. 1). The climate from the Lower Rio Grande Valley of Texas was the best match indicating that *E. hayati* should be the first candidate for evaluation as a biological control agent.

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Fig 1. Maps show the climatic similarity of the Lower Rio Grande Valley, TX; San Joaquin Valley, CA; and Imperial Valley, CA with Australia. Circles represent the climatic match with larger circles indicating a suitability of 70 % or better. The numbers represent the regions in Australia impacted by *Bemisia tabaci*: 1) Bowen/Burdekin, Queensland, 2) Central Highlands, Queensland, 3) Bundaberg, Queensland, 4) Lockyer Valley, Queensland, and 5) North-coastal, New South Wales.

Table 1.

Pre-release quarantine evaluation of *Bemisia tabaci* parasitoids in broccoli trials, based on Goolsby *et al.* (1998).

Table 2.

Comparison of *Eretmocerus* spp. establishment with matching of climates. For parasitoid establishment 1 = most commonly collected, no estab = no long term record of establishment. For CLIMEX ranking 1 = most similar climate followed by index of similarity. Recoveries indicate individuals that were collected in field samples and identified using both morphological and molecular techniques.

Species Name	Quarantine Accession No.*	Collection Locality	Number of nymphs parasitized per lifetime +/- SE**
<i>Eretmocerus mundus</i>	M92014	Spain, Murcia	63.0 +/- 5.6a
<i>Eretmocerus hayati</i>	M95012	Pakistan, Multan	43.6 +/- 6.1b
<i>Eretmocerus hayati</i>	M93005	India, Thirumala	43.2 +/- 7.9b
<i>Eretmocerus mundus</i>	M94092	Italy, Castel Gondolfo	41.0 +/- 7.4b
<i>Encarsia</i> sp. (<i>parvella</i> group)	M95001	Dominican Republic, Azua	36.4 +/- 6.2bc
<i>Eretmocerus mundus</i>	M94120	Israel, Golan Heights	34.0 +/- 5.4bcd
<i>Eretmocerus mundus</i>	M92019	India, Padappai	29.9 +/- 6.1bcd
<i>Eretmocerus melanoscutus</i>	M94040	Thailand, Kampang Saen	29.3 +/- 6.7bcd
<i>Encarsia pergandiella</i>	Local native	USA, Texas, Mission	24.0 +/- 4.7cde
<i>Eretmocerus tejanus</i>	Local native	USA, Texas, Mission	19.4 +/- 6.2def
<i>Encarsia</i> nr. <i>hispidula</i>	M94056	Brazil, Sete Lagoas	18.2 +/- 4.7defg
<i>Eretmocerus emiratus</i>	M95104	United Arab Emirates	11.9 +/- 4.4efgh
<i>Eretmocerus furuhashii</i>	M95026	Taiwan, Chiuju	10.9 +/- 3.6efgh
<i>Encarsia</i> nr. <i>pergandiella</i>	M94055	Brazil, Sete Lagoas	10.5 +/- 4.1efgh
<i>Encarsia lutea</i>	M94107	Israel, Givat Haim	7.9 +/- 2.7efgh
<i>Encarsia sophia</i>	M93003	Spain, Murcia	6.8 +/- 2.6fgh
<i>Encarsia sophia</i>	M94047	Malaysia, Kuala Lumpur	4.9 +/- 1.6fgh
<i>Eretmocerus melanoscutus</i>	M95097	Taiwan, Tainan	2.1 +/- 1.6gh
<i>Encarsia sophia</i>	M94016	Taiwan, Shan-Hua	1.6 +/- 0.9gh
<i>Eretmocerus staufferi</i>	M94002	USA, Texas (greenhouse)	0.2 +/- 0.2h

*Quarantine accession number begins with facility initial 'M' for Mission, TX followed by the year of importation and the sequential number assigned to each field collection.

**Means within rows followed by the same letter are not significantly different (P = 0.05).

San Joaquin Valley, CA

Species	Origin	Recoveries	Actual	CLIMEX	CLIMEX
		2002-2003	Establishment Rank	Ranking	Index of Similarity
<i>E. emiratus</i>	United Arab Emirates, Hormuz	0	no estab	3	68
<i>Eretmocerus</i> sp.	Ethiopia, Melka Werer*	0	no estab	2	74
<i>E. hayati</i>	Pakistan, Multan	0	no estab	4	64
<i>E. mundus</i>	Spain, Murcia	149	1	1	86
<i>E. melanoscutus</i>	Thailand, Chiang Mai	0	no estab	5	45

Rio Grande Valley, TX

Species	Origin	Recoveries	Actual	CLIMEX	CLIMEX
		1997-2002	Establishment Rank	Ranking	Index of Similarity
<i>E. emiratus</i>	United Arab Emirates, Hormuz	0	no estab	1	77
<i>Eretmocerus</i> sp.	Ethiopia, Melka Werer*	2	3	3	70
<i>E. hayati</i>	Pakistan, Multan	500	1	2	76
<i>E. mundus</i>	Spain, Murcia	5	2	4	68
<i>E. melanoscutus</i>	Thailand, Chiang Mai	0	no estab	5	60

Imperial Valley, CA

Species	Origin	Recoveries	Actual	CLIMEX	CLIMEX
		2001-2002	Establishment Rank	Ranking	Index of Similarity
<i>E. emiratus</i>	United Arab Emirates, Hormuz	4	2	2	79
<i>Eretmocerus</i> sp.	Ethiopia, Melka Werer*	57	1	4	72
<i>E. hayati</i>	Pakistan, Multan	0	no estab	1	80
<i>E. mundus</i>	Spain, Murcia	4	2	3	73

<i>E. melanoscutus</i>	Thailand, Chiang Mai	0	no estab	5	52
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Yuma, AZ

Species	Origin	Recoveries 2001-2002	Actual Establishment Rank	CLIMEX Ranking	CLIMEX Index of Similarity
<i>E. emiratus</i>	United Arab Emirates, Hormuz	0	no estab	1	80
<i>Eretmocerus</i> sp.	Ethiopia, Melka Werer*	50	1	3	73
<i>E. hayati</i>	Pakistan, Multan	0	no estab	1	80
<i>E. mundus</i>	Spain, Murcia	0	no estab	3	73
<i>E. melanoscutus</i>	Thailand, Chiang Mai	0	no estab	5	53

*Climatic comparison for Ethiopia uses only data from April 15- October 15 to account for its position on the equator.