CHAPTER EIGHT

Exploratory survey for natural enemies of *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) in India and climate matching to guide their introduction into Africa

ABSTRACT

The mango mealybug, *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) is one of the most destructive mealybug pest on several horticultural crops in Africa, mostly Tanzania, Kenya and Malawi where it continues to expand its host range. Although several indigenous parasitoid species have been found to attack the pest in both Kenya and Tanzania, their combined effort is unable to suppress the exploding populations of the mealybug pest below economically damaging levels. Being an alien invasive pest, classical biological control is mostly likely the optimal option for suppressing the population outbreaks in Africa. This prompted an exploratory survey in the aboriginal home of the pest across fifteen major horticultural production districts in the state of Tamil Nadu, India to identify efficient coevolved natural enemies for introduction into Africa. Based on the exploratory survey data, two correlative approaches to the challenge of ecological niche modeling (genetic algorithm and maximum entropy) were used to identify climatically suitable areas in Africa that are agro-meteorologically similar to the aboriginal home of the pest, based on associations between known occurrence records and a set of environmental predictor variables. Our results showed that *R. iceryoides* is widely distributed throughout the state of Tamil Nadu and was recorded from ten cultivated and non-cultivated host plant species from 8 different families with extremely low infestation levels. The combined percent parasitism based on the proportion of mummified mealybugs ranged between 16.67 to 91.3% on the different host plant parts. A total of eleven parasitoid species were recovered from *R. iceryoides*, out of which eight species are new records. *Praleurocerus viridis* Agarwal (Hymenoptera: Encyrtidae) and *Anagyrus chryos* Noyes & Hayat (Hymenoptera: Encyrtidae) were the dominant and most widely distributed species with maximum percentage parasitism of 43 and 41, respectively. In addition to the parasitoids, 10 predators from 7 families were recorded. The results of this work suggested that the concerted action of the native natural enemies was highly successful and quite effective in suppressing *R. iceryoides* populations. The two models yielded similar estimates, largely corresponding to Equatorial climate classes with temperature seasonality contributing the most in the ecological model with the highest predicted suitability for the pest and parasitoids. The maximum entropy approach was somewhat more conservative in its evaluation of suitability, depending on thresholds for presence/absence that are selected, largely excluding areas with distinct dry seasons; the genetic algorithm models, in contrast, indicate that climate class as partly suitable. The models parameters derived from India fitted well with the introduced distributional range of the pest in Africa and strongly suggest that the humid tropical coastlines of Kenya and Tanzania are climatically suitable for introduction. In addition to informing risk assessments for accidental introductions of the invasive pest, this prediction can also be used to focus monitoring activities.

Keywords: Mango mealybug, *Rastrococcus iceryoides*, foreign exploration, natural enemies, climate matching, Classical biological control, GARP, Maxent

8.1 Introduction

The invasive mango mealybug, *R. iceryoides* (Green) (Homoptera: Pseudococcidae), is a pest of major economic importance in East Africa (Williams, 1989). This insect was first reported in the early 1990s damaging fruit, twigs and foliage of mango in Tanzania. It has since spread inland to northern Malawian boarder and Coastal Kenya and has become a major pest of mango in these countries as well (Neuenschwander, 1993; Tanga, unpublished data). Heavily infested orchards in Tanzania and Kenya were observed to experience damage ranging from 30% to complete crop failure and fruits that suffered feeding damage are either unmarketable or downgraded in packing houses. Feeding damage on the plant results in defoliation, early dropping of the inflorescence and young fruits as well as severe dieback effect of affected young branches. As with other mealybug species, severe infestation on fresh mango fruits can cause a significant reduction in weight and size (Tobih et al., 2002; Pitan et al., 2002; Tanga et al., unpublished data).

The current control methods by majority of the growers to suppress *R. iceryoides* is cutting down and burning of infested trees in addition to aggressive broad insecticidal canopy sprays (Tanga et al., unpublished data; Association of Mango Growers Association of Tanzania, pers. comm.). Although, chemical control is largely practiced in Tanzania and Kenya, it is ineffective because of the waxy coating of mealybugs. The introduction of strict maximum residue level by the EU has further compounded the overuse of chemical pesticide application. The increase and ineffective use of chemical insecticide is likely to jeopardize resistance development as has been reported for other mealybug species (Myburgh and Siebert, 1964; Flaherty et al., 1982).

Biological control with natural enemies is most recommended of mealybug management (Norgaard, 1988; Agounké and Fischer, 1993; Kairo et al., 2000; Moore, 2004; Roltsch et al., 2006; Garcia-Valente et al., 2009). In a recent survey (Chapter three), although up to six parasitoid species (*Anagyrus pseudococci* Girault, *Anagyrus aegyptiacus* Moursi, *Leptomastrix dactylopii* Howard, *Agarwalencyrtus citri* Agarwal, *Aenasius longiscapus* Compere and *Leptomastidea tecta* Prinsloo) were recovered from *R. iceryoides* (some representing new associations) only *A. pseudococci* was able to cause up to 21% parasitism. Parasitism by the other parasitoid species encountered during the survey did not exceed 1%. Despite the relatively high levels of parasitism by *A. pseudococci*, the ability of the parasitoid to regulate the population of *R. iceryoides* in Tanzania and Kenya has been inadequate. In its native home range (Southern Asia), Tandon and Lal (1978), Narasimham and Chako, (1988), and Tandon and Srivastava (1980) reported that *R. iceryoides* is attacked by several parasitoid species (*Tetrastichus* sp., *Allotropa* sp., *Anagyrus* sp. nr *inopus* Noyes & Hayat, *Praleurocerus viridis* (Agarwal), *Coccophagus* sp., *Promuscidea unfasciativentris* Girault and *Chartocerus* sp) with parasitism rates exceeding 40%. This high level of parasitism renders the pest to be of no economic significance in that region. Interestingly, in spite of the rising importance and high levels of damage by *R. iceryoides* to Agriculture in Africa no natural enemies have been introduced so far into Africa to manage the pest.

Biological control through the introduction of natural enemies has been practiced for over 100 years and it is believed to be the only long-term sustainable solution to the problems caused by exotic pests. However, much remains to be discovered about the factors contributing to the success or failure of introductions. About two thirds of natural enemies introduced have failed to establish (Hall and Ehler, 1979; Stiling, 1990), and only about half of the introductions that established against arthropod pests provided some level of economic control. These failures have often been attributed to a lack of climatic matching between area-of-origin and areas-of-establishment (Clausen, 1978; Stiling, 1993; Goolsby et al., 2005). It has therefore been proposed that biological control needs to make the transition from an empirical method to climatic predictive science (Greathead, 1986; Ehler, 1990; Hoddle and Syrett, 2002). This is because climate is an important factor in the bio-ecology of insect natural enemies and a useful predictor of potential establishment and spread in areas of introduction (Cammell and Knight, 1992; Gevrey and Worner, 2006; Kiritani, 2006; Tuda et al., 2006; Guo et al., 2006; Hance et al., 2007). In this regard, is it now widely recommended that natural enemies should be collected from climates that closely match the environment into which they will be introduced (Stiling, 1993; Sutherst, 2003; Hoelmer and Kirk, 2005). Current reviews of invasive species biology have emphasized the great complexities involved in species' occupancy of new distributional areas (Carlton, 1996; NAS, 2002). However, advances in the emerging field of ecological niche modeling have opened the possibility of using species' ecological characteristics as evaluated on native distributional areas to predict potential distributional areas in other regions (Higgins et al., 1999; Skov, 2000; Zalba et al., 2000; Hoffmann, 2001), given the precept that species' ecological niche characteristics tend to remain fairly constant (Peterson, 2003) although some examples of plasticity have been documented (Maron et al., 2004). Predictive modeling of species geographic distributions based on the environmental variables of sites of known occurrence constitutes are now widely used to predict potential establishment and distributional ranges of introduced natural enemies (Yom-Tov and Kadmon, 1998; Peterson et al., 1999; Welk et al., 2002; Scott et al., 2002; Peterson and Shaw, 2003).

This investigation establishes the patterns of *R. iceryoides* distribution, host plant relationships and its associated coevolved natural enemies in southern Indian State of Tamil Nadu with the aim of selecting candidate natural enemies that could be introduced into Africa for the management of the pest. Ecological niche modeling was applied to better understand the global range of expansion of *R. iceryoides* and suitable climatic conditions and localities for introduction and potential for establishment of the natural enemies in Africa.

8.2 Materials and Methods

8.2.1 Sampling sites

India is the largest mango producer in the world accounting for about 40% of the global production (Hanemann, 2006). Within the country, Tamil Nadu is considered as one of the most important mango production states both in terms of quantity and commercial utilization (Mehta and George, 2003; Tharanathan et al., 2006) with 385 fruit processing units (Raj, 2008). The sampling sites were chosen based on this previous knowledge of mango and other horticultural production. The field surveys were conducted in 15 major mango growing districts of Tamil Nadu State.

8.2.2 Plant collection, handling and assessment of infestation

The survey methodology was a slight modification from that described by Pitan et al. (2000) and Bokonon-Ganta and Neuenschwander (1995). The procedure was based on an unbiased choice of sample locations along footpaths and jeep trails in major mango production localities of the state of Tamil Nadu. Sampling was carried out in cultivated fields, backyard gardens, woodlands, high-ways, intra-city roads, motorable village roads, forested areas and protected reserves. At each location, a 6-10 km transect was set up with sampling points at 0 km, 2 km, 4 km, 6 km, 8 km and 10 km from the most northerly point of the transect. At each of the sampling points along the transect host plants were selected using random bearings from which 80 leaves, 20 (10-cm length) twigs and five fruits were selected for mealybug counts. Sampling along the transect leading away from the locations was discontinued after several stops without *R. iceryoides* infestation (Bokonon-Ganta and Neuenschwander, 1995). The GPS (global poistioning system) readings were recorded for all the sampled points within each area surveyed. Plant parts were individually transferred to paper bags and transported to the laboratory in cool boxes. In the laboratory, the total number of *R. iceryoides* per sampled plants parts were counted with the aid of a head lens and or stereomicroscope, and recorded. The severity of mealybug infestation was scored for each locality and host plant from the infested foliage, twigs, and fruits following the scale developed by Tobih et al. (2002) for *R. invadens*. Infestation by *R. iceryoides* was also expressed as the total number of female mealybugs per plant part sampled for each locality.

From the field collected mealybug, five to ten adult mealybug samples were randomly selected and slide-mounted at the Departments of Agricultural Entomology, Centre for Plant Protection Studies (CPPS), Tamil Nadu Agricultural University (TNAU) using the methodology of Watson and Kubiriba (2005), for further confirmation of their identity. Voucher specimens for collected mealybug samples were deposited at CPPS, Tamil Nadu Agricultural University Unit. Samples of leaf and or twig and fruit (for small fruit) from unknown plant species were collected, pressed and bagged. The collected plant samples were identified using a field key to the trees and lianas of the evergreen forest of India (Bole and Vaghani, 1986; Pascal and Ramesh, 1997). Photographs were also taken of each plant and or fruit sampled to aid in plant identification and voucher specimens of all collections of the plant species are maintained in the above institution. Plant nomenclature used conforms to the International Plant Names Index database (IPNI, 2005) and the Missouri Botanical Garden database W^3 TROPICOS (MBOT, 2006).

8.2.3 Parasitoid and predator recovery from field collected mealybug samples

After the census of mealybugs on infested plant parts, live and mummified specimen were transferred into plastic paper bags with well ventilated tiny openings made using entomological pins # 000 (length 38 mm, 0.25 mm diameter) or transparent plastic rearing containers (22.5 cm height x 20 cm top diameter x 15 cm bottom diameter). An opening (10 cm diameter) was made on the front side of the cage to which a sleeve, made from very fine organza material (about 0.1 mm mesh size) was fixed. The same material was fixed to the opposite opening (10 cm diameter) of the cage to allow for ventilation. A third opening (13 cm diameter) was made on the roof of the cage, which was also screened with the same material. Streaks of undiluted honey were applied to the roof of the cages and maintained in the laboratory at 70 ± 5% RH, photoperiod of 12:12 (L: D) h and ambient temperatures (26-28^{0}C) until parasitoid emergence. Mummies with emergence holes were discarded after counting. Mummified mealybugs from each infested host plant species and locality were maintained separately. Parasitoids that emerged from the mealybug cultures were collected daily and counted. All parasitoids that emerged were initially identified by Dr. Sagadai Manickavasagam at Annamalai University, India and later confirmed at the Department of Zoology, Aligarh Muslim University, India by Dr. Hayat Mohammad. Voucher specimens of parasitoids of the present study were deposited in the collections of Project Directorate of Biological Control (PDBC), Aligarh Muslim University (AMU), Bangalore; Departments of Agricultural Entomology, Centre for Plant Protection Studies (CPPS), Tamil Nadu Agricultural University (TNAU) and in Annamalai University, Faculty of Agriculture.

At each sampling date and site, predators of *R. iceryoides* were sampled by beating 10 randomly selected branches of each host plants over a 1 – m^2 cloth screen using a 60 cm long stick. The sampling was done during the early hours of the morning of 8:30-9:30 am. The predators that were dislodged onto the cloth were then recorded and preserved in 70 % ethyl alcohol. Immature stages of predators were reared on mealybugs in transparent plastic rearing containers (22 cm length x 15 cm width x 15 cm height) with an opening (10 cm diameter) made on the front side of the plastic container to which a sleeve, made of organza material was fixed. The set up was maintained at 26-28˚C, 40 - 80% relative humidity (RH), under a photoperiod of 12L: 12D in the laboratory at the Departments of Agricultural Entomology, Tamil Nadu

Agricultural University (TNAU), until they developed to the adult stage and later counted. The interactions between ant and mealybug populations in the field were randomly assessed by means of visual inspection.

8.2.4 Statistical Analysis

Data for field surveys are presented according to plant species, family, location, infestation levels, combined percentage parasitism based on the proportion of mummified mealybug and the number of emerged adult parasitoids. Infestation by *R. iceryoides* was expressed as the total number of mealybugs of all developmental stages per plant part sampled for each locality. Parasitism was expressed as percentage of mummified mealybugs collected or as percentage of emerged parasitoid species to the total number of hosts in the samples for each host plant per locality. The data on mealybug incidences and percentage parasitism were compared across plant parts by subjecting the data to Wilcoxon-Mann-Whitney two sample signed rank test and Kruskal-Wallis test to detect differences (Conover, 1999). All computations were performed using SAS 9.1 software (SAS Institute, 2010).

8.2.5 Climate matching models and computation of climate suitability

The basic concept underlying species occurrence modelling is the definition of the climate niche: each species occurs within specific ranges of environmental variables, enabling individuals to survive and reproduce (Austin, 2002). The most common strategy for estimating the potential geographic distribution of a species is to characterize the environmental conditions that are suitable for that species. The spatial distribution of environments that are suitable for a species can then be estimated across a given study region. A wide variety of modeling techniques have been developed for this purpose, including generalized linear models, generalized additive models, bioclimatic envelopes, habitat suitability indices, and the genetic algorithm for rule-set prediction (GARP). In this study, two correlative approaches namely Maxent (maximum entropy modeling; Phillips et al., 2006), and the OM-GARP (genetic algorithm for rule-set prediction; Stockwell and Noble, 1992) were used. These algorithms produce predictions from incomplete information by estimating the most uniform distribution of points of occurrence across the area of study. The evaluation of performance of the models is also carried out to determine the significance of each individual climate variable and provides a measure of the accuracy of the model. This procedure allows for the selection of the final set of significant variables. Calculation of the basic climate niche for each species is an adequate framework to understand the climate-derived drivers of climate suitability.

8.2.5.1 Occurrence data of *R. iceryoides*

Native records of *R. iceryoides* in the state of Tamil Nadu, India and non-native distribution in Africa (particularly Kenya and Tanzania) are summarized in Figure 8.1, resulting from independent surveys conducted by the authors in Kenya, Tanzania and India (C.M. Tanga et al., unpublished data). All records are based upon specimens clearly identified as *R. iceryoides* and differentiated from other taxa within the genus *Rastrococcus*. This list is far from being exhaustive, in the sense that it comprises only data from our survey excluding all extensively unpublished data. The non-native data enable quantitative tests of the predictive ability of the ecological niche models regarding the geographic potential of the species. Only occurrence data originating from the species' native distribution observed during our survey was used to generate the ecological niche modeling.

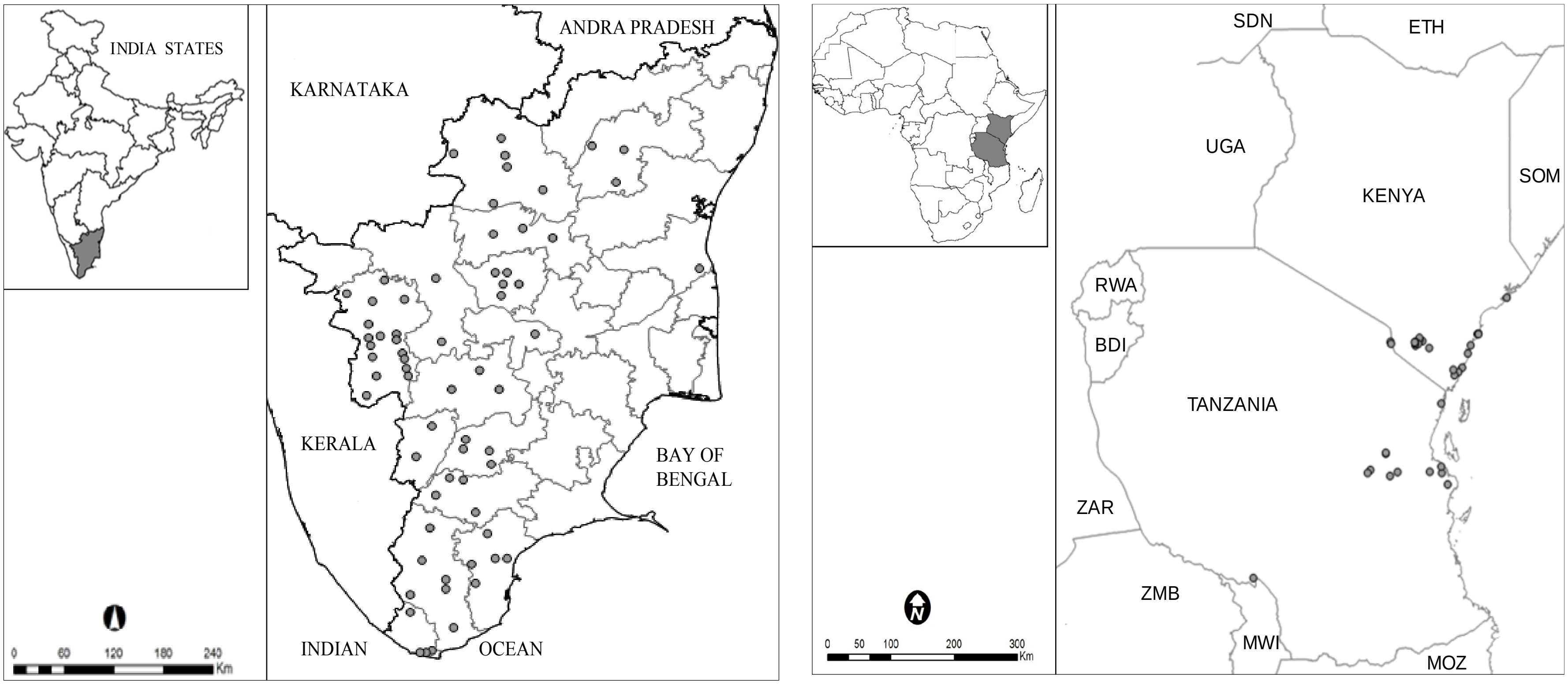

Figure 8. 1: Native records of *R. iceryoides* in the state of Tamil Nadu, India. and which were used in GARP and Maxent to determine climatically similar areas of India, Africa and the world. Non-native records in Africa (Kenya and Tanzania).

8.2.5.2 Environmental data

A set of 19 aggregated bioclimatic variables averaged over a 50-year time period from 1950–2000 at 2.5 arc-minutes spatial resolution were downloaded from www.worldclim.org. These particular climate dimensions were chosen to represent environmental dimensions relevant to distributions and survival of small arthropods (Fletcher, 1989; Vargas et al., 1987; Vera et al., 2002), of which mealybugs are inclusive. No vegetation or land cover data layers were used owing to the heterogeneous nature of habitats, including man-made horticultural environments that can potentially be occupied by *R. iceryoides*. Although host range can provide useful information with regard to species recognition, this information remains incomplete for *R. iceryoides*, particularly as regards the non-native range.

8.2.5.3 Ecological niche modeling (ENM)

The approach used was based on the idea of modeling species' ecological niches, which are considered to constitute long-term stable constraints on species' potential geographic distributions (Peterson et al., 1999; Peterson, 2003; Raxworthy et al., 2003; Martinez-Meyer et al., 2004; Wiens and Graham, 2005). Ecological niches are herein defined as the set of conditions under which a species is able to maintain populations without immigration (Grinnell, 1917; Grinnell, 1924). This condition is assumed here although the species is an extraordinary poorly known one, in particular in its non-native range in Africa. Our approach consisted of four steps: (1) model ecological niche requirements of the species based on known occurrences on its native distributional area during our survey; (2) project niche models to global scales to identify areas fitting the niche profile, (3) test the accuracy of native and invaded range predictions, and (4) project the niche model globally to identify additional areas putatively susceptible to invasion. The global projection was based on a niche model trained using all the native and non-native range records.

The inferential tools used for the ecological niche modeling were GARP and Maxent, both on default settings. These two techniques provided contrasting results in recent comparisons of niche modeling techniques (Elith et al., 2006; Peterson et al., 2007; Peterson et al., 2008). GARP uses an evolutionary-computing approach to carry out a flexible and powerful search for non-random associations between environmental variables and known occurrences of species.

GARP has been used widely (Peterson, 2001; Peterson, 2005; Anderson et al., 2002; Anderson et al., 2003; Stockwell and Peterson, 2002). Specifically, available occurrence points are sub-sampled to create two suites of points: half of the available points are set aside as extrinsic testing points; the remaining points are then re-sampled with replacement to create a population of 1250 presence points; an equivalent number of points is re-sampled from the population of grid squares ('pixels') from which the species has not been recorded ('pseu-doabsence data'). These 2500 points are divided equally into training (for creating models) and intrinsic testing (for evaluating model quality) data sets. Models are composed of a set of conditional rules developed through an iterative process of rule selection, evaluation, testing, and incorporation or rejection. First, a method is chosen from a set of possibilities (e.g. logistic regression, bioclimatic rules, range rules etc), and applied to the training data set. Then, rules 'evolve' by a number of means (mimicking DNA evolution: point mutations, deletions, crossing over, etc.) to maximize predictive accuracy. After each modification, rule quality is evaluated based on the intrinsic testing data; change in predictive accuracy from one iteration to the next is used to evaluate whether a particular rule should be incorporated into the final rule-set. The algorithm runs either 1,000 iterations or until addition of new rules has no effect on predictive accuracy. The final rule-set (the ecological niche model) is then projected worldwide to identify a potential geographic distribution. In general, all analyses were run on default settings, and the best-subsets procedure (Anderson et al., 2003; Rice et al., 2003) was used to choose a subset of models for further consideration, which were then summed to produce a single grid summarizing model agreement in predicting presence vs. absence. This grid was converted to a binary prediction of presence vs. absence by choosing the lowest threshold at which the species was known to occur (Pearson et al., 2007). The result was a set of binary grids summarizing the geographic extents of the environmental niche calculated by GARP for the species.

Maxent makes use of presence records and a set of background values (pseudo-absences) drawn from the entire study region. However, habitat suitability models are calculated only with presence data and predict the species' fundamental niche (i.e., the full range of abiotic conditions within which the species is viable), such that the model output is the probability of a particular habitat to be suitable for the species' survival (Brito and Crespo, 2002). The maximum entropy algorithm (Phillips et al., 2006) for species distributions modeling is one of the most accurate and globally used ecological niche models (Hernandez et al., 2006). Maxent besides generating accurate models, provides an output which identifies the role of each environmental variable in the prediction model. Maxent is a generative approach, rather than discriminative, which can be an inherent advantage when the amount of training data is limited. Maxent estimates the ecological niche of a species by determining the distribution of maximum entropy, subject to the constraint that the expected value of each environmental variable under this estimated distribution matches its empirical average (Phillips et al., 2006). We used default parameters in Maxent (version 3.1) to produce models: feature selection automatic, regularization multiplier at unity, maximum iterations 500, convergence threshold 10^{-5} and random test percentage at zero. The result is a set of probabilities that sum to unity across the entire study area; to make values more manageable, these suitability indices are usually presented as logistic transformations of cumulative probabilities (Phillips et al., 2006), with values ranging 0–100 (low to high suitability).

Spatial predictions of presence and absence can include two types of error, omission (predicted absence in areas of actual presence) and commission (predicted presence in areas of actual absence: Fielding and Bell, 1997). Because GARP is a random-walk procedure, it does not produce unique solutions; consequently, we followed best-practices approaches to identifying optimal subsets of resulting replicate models (Anderson et al., 2003). In particular, we developed 100 replicate models; of these models, we retained the 20 with lowest extrinsic omission error rates and then retained the ten models with intermediate extrinsic commission error (i.e. we discarded the ten models with area predicted present showing greatest deviations from the overall median area predicted present across all low omission models). This 'best subset' of models was summed pixel by pixel to produce final predictions of potential distributions in the form of grids with values ranging from 0 (all models agree in predicting absence) to 10 (all models agree in predicting presence). Since the two modeling techniques produce different sorts of output with very different frequency distributions, correct choice of thresholds becomes critical in interpreting the resulting maps (Peterson et al., 2007). As such, we used the lowest training presence threshold approach (LTPT) of Pearson et al. (2007); specifically, we inspected the native-range occurrence information relative to the raw outputs from GARP and Maxent. We determined the lowest predictive level at which any training presence point was predicted and used that level as a minimum criterion for prediction of presence (vs. absence) in non-native regions.

8.2.5.4 Model testing

To evaluate the model predictions, we offer two sets of tests. First, we developed initial models across the native range region based on a subset of available data, in which ten randomly chosen points were set aside (for testing) prior to model development; this procedure was repeated twice, with different random subsamples. Statistical significance of these predictions was assessed using the cumulative binomial probability approach described below. Second, we assessed the predictive ability in Africa (using African records) for a model that was calibrated using all records from the native region. Because our goal was predicting global invasive potential, we tested model predictivity with the null hypothesis that the observed coincidence between prediction and test points was no better than chance expectations.

The most common mode of evaluating niche models in recent literature is via the area under the curve in a receiver operating characteristic (ROC) analysis (e.g., Elith et al., 2006). ROC analysis, however, is not appropriate to the present situation for two reasons: (i) ROCs require absence data, which are not available in the present case; and (ii) ROCs weight type 1 and type 2 errors equally, but the focus on invasive potential would weight omission error more heavily than commission error (Soberon and Peterson, 2005; Peterson et al., 2008). However, we use an adaptation of the ROC curve approach as a means of assessing predictive ability visually, plotting omission on an inverse scale (='sensitivity') against proportion of area predicted present (an estimator of 1–specificity: Phillips et al., 2006; Peterson et al., 2008).

8.3 Results

8.3.1 Distribution

In the state of Tamil Nadu, out of the 15 districts sampled, *R. iceryoides* was recorded from all the localities of the districts, but with varying degree of infestation (Table 8.1). Among all the locations sampled, infestation was heaviest on mango in Kundal village found in the district of Kanyakumari with 9.5 ± 3.0 mealybugs/leaf, 45.4 ± 15.2 mealybugs/twig and 73.8 ± 50.1 mealybugs/fruit, followed by Pechiparai in the same district with 2.0 ± 0.8 mealybugs/leaf and 22.4 ± 7.1 mealybugs/twig. The lowest infestation level on mango was recorded in the district of Erode with 0.1 ± 0.1 mealybugs/leaf and 0.8 ± 0.7 mealybugs/twig.

8.3.2 Host-plants

During the survey, *R. iceryoides* was recorded from ten cultivated and wild host plants belonging to eight different families with extremely low levels of infestation (Table 8.1). The host plants included *Mangifera indica* L. [Anacardiaceae], *Manilkara zapota* L. [Sapotaceae], *Tectona grandis* L. [Verbenaceae], *Ficus benghalensis* L. [Moraceae], *Gossypium hirsutum* L. [Malvaceae], *Gossypium gossypioides* Ulbr. [Malvaceae], *Pongamia pinnata* L. [Fabaceae], *Psidium guajava* L. [Myrtaceae], *Cajanus cajan* (L) Millsp. [Fabaceae], *Ceiba pentandra* L. [Bombacaceae]. The highest infestation levels were recorded on the twigs (45.4 ± 15.2 mealybugs/twig), leaves (9.5 ± 3.0 mealybugs/leaf) and fruits (73.8 ± 50.1 mealybugs/fruit) of *M. indica* in Kundal village, Kanyakumari district. *Psidium guajava* was the second most infested cultivated host plant (1.69 ± 0.80 mealybugs/leaf and 4.60 ± 2.17 mealybugs/twig), followed by *M. zapota* (1.96 ± 1.05 mealybugs/leaf and 3.90 ± 2.26 mealybugs/twig) in Coimbatore.

However, there was no significant difference in the infestation levels between leaves and twigs of the different host plants sampled during the survey except for mango in Paiyur (Mann-Whitney test, Z = -2.1632; df = 1; P = 0.0305). On mango, the fruits recorded the highest number of mealybugs although no significant differences was observed when compared to those on the twigs and leaves (Kruskal-Wallis test, χ^2 = 2.7027; df = 2; P = 0.2589) (Table 8.1). In Coimbatore, where the second highest level of infestation on mango was recorded, the mealybug population on the twigs were higher than on the leaves but not significantly different (Mann-Whitney test, Z = 0.2196; df = 1; P = 0.8262). The most important wild host plant was *F. benghalensis* with infestation levels of 2.0 ± 1.07 mealybugs/leaf and 2.7 ± 1.3 mealybugs/twig. However, the number of mealybug on the twig of *F. benghalensis* was not significantly different from that on the leaves (Mann-Whitney test, Z = -0.2299; df = 1; P = 0.2187) (Table 8.1).

Other mealybug species were also encountered, although at negligible level on mango, these include: *R. invadens* (William) found in two localities only (Coimbatore and Madurai) from two host plants (*M. indica* and *M. zapota*); *Drosicha mangiferae* (Green) found in Paiyur only; *Icerya aegyptiaca* (Douglas) and *Icerya seychellarum* (Westwood), which were restricted to Coimbator; *Planococcus citri* (Risso), *Ferrisia virgata* (Cockerell) and *Paracoccus marginetus* (Williams and Granara de Willink) [important on *Carica papaya* Linn. (Family Caricaceae)], *Plumeria alba* Linn. (Family: Apocynaceae), *Solanum torvum* Swartz (Family: Solanaceae) and *P. guajava* (Family: Myrtaceae)].

Table 8. 1: Distribution, host plants and infestation levels of *R. iceryoides* in the state of Tamil Nadu, India

District/Locality	Host plants	Plant family	Mean no. of mealybugs/leaf	Mean no. of mealybugs/twig	Mean no. of mealybugs/fruit	Statistics		
						Z/χ2	*df*	*P*
Coimbatore	*Mangifera indica* Linn.	Anacardiaceae	3.70 ± 1.74	9.15 ± 4.43	-	0.2196	1	0.8262
	Manilkara zapota Linn.	Sapotaceae	1.96 ± 1.05	3.90 ± 2.26	-	-0.6124	1	0.5403
	Tectona grandis Linn.	Verbenaceae	0.68 ± 0.36	2.0 ± 1.12	-	0.5238	1	0.6004
	Ficus benghalensis Linn.	Moraceae	2.03 ± 1.07	2.7 ± 1.34	-	-0.2299	1	0.2187
	Gossypium hirsutum Linn.	Malvaceae	0.25 ± 0.16	0.30 ± 0.21	-	-0.9172	1	0.3590
	Gossypium gossypioides Ulbr.	Malvaceae	0.38 ± 0.20	0.90 ± 0.50	-	1.1616	1	0.2454
	Pongamia pinnata Linn.	Fabaceae	1.09 ± 0.59	1.85 ± 1.12	-	-0.5490	1	0.5830
	Psidium guajava Linn.	Myrtaceae	1.69 ± 0.80	4.60 ± 2.17	-	-0.2861	1	0.7748
	Cajanus cajan (L) Millsp.	Fabaceae	0.29 ± 0.22	0.70 ± 0.42	-	0.3568	1	0.7213
Salem								
Erummaiputti	*Mangifera indica* Linn.	Anacardiaceae	2.78 ± 1.33	7.10 ± 3.23	-	-0.3571	1	0.7210
Dharmapuri								
Paiyur	*Mangifera indica* Linn.	Anacardiaceae	3.21 ± 1.24	1.40 ± 0.98	-	-2.1632	1	0.0305
	Ceiba pentandra Linn.	Bombacaceae	0.54 ± 0.27	0.60 ± 0.34	-	-1.4759	1	0.1400
Periyapatti	*Mangifera indica* Linn.	Anacardiaceae	4.61 ± 1.96	5.75 ± 2.43	-	-1.1216	1	0.2620
	Cajanus cajan (L) Millsp.	Fabaceae	0.86 ± 0.43	0.80 ± 0.52	-	-1.8371	1	0.0662
Kariyamangakam	*Mangifera indica* Linn.	Anacardiaceae	1.39 ± 0.63	3.25 ± 1.79	-	-0.5170	1	0.6052
Madurai								
Othakadei	*Mangifera indica* Linn.	Anacardiaceae	1.08 ± 0.52	1.15 ± 0.52	-	-1.3038	1	0.1923
	Ceiba pentandra Linn.	Bombacaceae	0.35 ± 0.20	0.35 ± 0.18	-	1.7530	1	0.0796
Valeyapatti	*Mangifera indica* Linn.	Anacardiaceae	0.64 ± 0.36	0.75 ± 0.32	-	1.9347	1	0.0530

Plants parts samples based on 80 leaves, 20 twigs of 10 cm length and 5 fruits; - = plant part were either not infested and omitted from analysis or not available during sampling.

Table 8.1 continues. Distribution, host plants and infestation levels of *R. iceryoides* in the state of Tamil Nadu, India

District/Locality	Host plants	Plant family	Mean no. of mealybugs/leaf	Mean no. of mealybugs/twig	Mean no. of mealybugs/fruit	Statistics		
						Z/χ2	*df*	*P*
Virudhunagar								
Rajapalayam	*Mangifera indica* Linn.	Anacardiaceae	0.81 ± 0.35	0.75 ± 0.44	-	-1.9246	1	0.0543
Theni								
Periyakulam	*Mangifera indica* Linn.	Anacardiaceae	0.43 ± 0.29	0.95 ± 0.50	-	0.3721	1	0.7098
Kanyakumari								
Pechiparai	*Mangifera indica* Linn.	Anacardiaceae	2.03 ± 0.80	22.35 ± 7.09	-	-1.6340	1	0.1022
Kundal	*Mangifera indica* Linn.	Anacardiaceae	9.46 ± 2.99	45.4 ± 15.23	73.8 ± 50.11	2.7027	2	0.2589
Erode	*Mangifera indica* Linn.	Anacardiaceae	0.13 ± 0.07	0.75 ± 0.56	-	0.1800	1	0.8571
	Ceiba pentandra Linn.	Bombacaceae	0.58 ± 0.26	1.60 ± 0.73	-	-0.2873	1	0.7739
Cuddalore	*Manilkara zapota* Linn.	Sapotaceae	0.50 ± 0.24	1.60 ± 0.76	-	0.3214	1	0.7479
Tirunelveli	*Mangifera indica* Linn.	Anacardiaceae	0.29 ± 0.15	1.40 ± 0.74	-	0.4920	1	0.6228
Tiruvannamalai	*Gossypium hirsutum* Linn.	Malvaceae	0.11 ± 0.07	1.0 ± 0.50	-	-0.3018	1	0.7628
Tuticorin	*Gossypium gossypioides* Ulbr.	Malvaceae	0.01 ± 0.01	0.95 ± 0.48	-	-1.0607	1	0.2888
Karur	*Psidium guajava* Linn.	Myrtaceae	0.43 ± 0.25	0.60 ± 0.42	-	-0.8839	1	0.3768
Dindigul	*Mangifera indica* Linn.	Anacardiaceae	0.23 ± 0.10	1.35 ± 0.81	-	1.4473	1	0.1478
Namakkal	*Mangifera indica* Linn.	Anacardiaceae	0.24 ± 0.12	0.65 ± 0.35	-	0.4871	1	0.6262

Plants parts samples based on 80 leaves, 20 twigs of 10 cm length and 5 fruits; - = plant part were either not infested and omitted from analysis or not available during sampling.

8.3.3 Parasitoids associated with *R. iceryoides* in the state of Tamil Nadu, India

Out of 5950 *R. iceryoides* collected from the eight host plant species across the 15 districts, 3788 mealybugs were parasitized and yielded a parasitism rate of 63.66%. Out of the total number of mummified mealybug collected from the different host plants, 3167 mummified mealybugs were from mango accounting for 83.61% of total mummified mealybugs. Combined parasitism rate based on proportion of mummified mealybugs varied across host plants as well as host plant parts (Table 8.2). The highest combined percentage parasitism was recorded on mango in Othakadei, Madurai district with 88.37% on the leaves and 91.3% on the twigs. The lowest combined percentage parasitism was recorded on *P. guajava* in Karur district with 20.59% on the leaves and 16.67% on the twigs. Combined percentage parasitism on the different plant parts across the different host plants generally showed insignificant relationships between leaves and the twigs except on mango in Paiyur, Dharmapuri district ($Z = 2.0888$; $df = 1$; $P = 0.0367$) and on *F. benghalensis* in Coimbatore ($Z = -2.3368$; $df = 1$; $P = 0.0195$). The parasitoid community was composed of eleven primary parasitoid species recovered from *R. iceryoides*, out of which seven are new records (Table 8.3). These parasitoid species were from the family Encyrtidae: *Praleurocerus viridis* Agarwal, *Anagyrus chryos* Noyes & Hayat, *Parechthrodryinus excelsus* Hayat, *Ericydnus paludatus* Haliday, *Neoplatycerus tachikawai* Subba Rao, *Agarwalencyrtus ajmerensis* Fatima & Shafee, *Aenasius advena* Compere, *Leptomastidea minyas* Noyes & Hayat, *Carabunia bicoloripes* Hayat, *Aphycus sapporoensis* Compere & Annecke and *Anagyrus mirzia* Agarwal & Alam (Figure 8.2). *Praleurocerus viridis* and *A. chryos* were the most abundant and widely distributed species during the survey accounted for 34.05 and 28.17% of total emerged parasitoids, respectively. The remaining nine parasitoid species comprised only 14.35% of the total wasps recovered. The percentage parasitism of the different parasitoid species varied considerably among the different host plant species (Table 8.3). For example, *P. viridis* achieved a maximum percentage parasitism of 43% on twigs of *C. pentandra* in Othakadei, Madurai district while *A. chryos* achieved a maximum percentage parasitism of 41% on leaves of the mango plant in Periyakulam, Theni district. The percentage parasitism of *P. viridis* and *A. chryos* recorded on mango fruit in Kundal village, Kanyakumari district ranged between 16 – 30%.

Two hyperparasitoid species were also recovered from *R. iceryoides* during the survey: *Coccophagus ceroplastae* (Howard) (Hymenoptera: Aphelinidae) and *Coccidoctonus terebratus*

(Hayat, Alam & Agarwal) (Hymenoptera: Encyrtidae) (Figure 8.3), with the later restricted to Salem only. Both hyperparasitoids accounted for 12.83% of total parasitoids recovered from *R. iceryoides* during the survey. *Coccophagus ceroplastae* was the most dominant hyperparasitoids accounting for a hyperparasitism rate of 2.7 - 11.11% on leaves and 0 - 13.33% on twigs across the different host plants sampled during the survey (Table 8.3). However, percentage hyperparasitism was sporadic and low on all sampled localities.

Other parasitoids recovered during the survey from different mealybug species included, a dipteran parasitoid species, *Cryptochaetum iceryae* (Williston) (Diptera: Cryptochaetidae) from *I. seychellarum* in Coimbatore. This parasitic fly was observed from 6 parasitized out of the 8 samples collected from the field accounting for 75% parasitism of *I. seychellarum*. *Aenasius advena* Compere (Hymenoptera: Encyrtidae) was recovered from *P. citri* and *F. virgata*. *Gyranusoidea tebygi* Noyes and *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae) were recovered from *R. invadens* in Coimbatore and Madurai districts. No parasitoids were recovered from *Drosicha mangiferae* and *Icerya aegyptiaca.*

Table 8. 2: Combined percentage parasitism based on the number of mummified *R. iceryoides* in the state of Tamil Nadu, India

		Percentage parasitism			Statistics		
District/Locality	Host plants	Leaves	Twigs	Fruits	Z/χ^2	*df*	*P*
Coimbatore	*Mangifera indica* Linn.	73.99 (296)	74.86 (183)	-	-0.4518	1	0.6514
	Manilkara zapota Linn.	40.76 (157)	43.59 (78)	-	0.2745	1	0.7837
	Tectona grandis Linn.	55.56 (54)	55.0 (40)	-	-0.1235	1	0.9017
	Ficus benghalensis Linn.	57.41 (162)	20.37 (54)	-	-2.3368	1	0.0195
	Gossypium hirsutum Linn.	25.0 (20)	50.0 (6)	-	0.5401	1	0.5892
	Gossypium gossypioides Ulbr.	30.0 (30)	44.44 (18)	-	-0.5843	1	0.5590
	Pongamia pinnata Linn.	47.13 (87)	70.27 (37)	-	1.3858	1	0.1658
	Psidium guajava Linn.	59.26 (135)	33.7 (92)	-	-1.3571	1	0.1747
	Cajanus cajan (L) Millsp.	34.78 (23)	35.71 (14)	-	0.1768	1	0.8597
Salem							
Erummaiputti	*Mangifera indica* Linn.	82.88 (222)	78.87 (142)	-	-0.7153	1	0.4744
Dharmapuri							
Paiyur	*Mangifera indica* Linn.	77.04 (257)	89.29 (28)	-	2.0888	1	0.0367
	Ceiba pentandra Linn.	34.88 (43)	41.67 (12)	-	0.1246	1	0.9009
Periyapatti	*Mangifera indica* Linn.	81.3 (369)	85.22 (115)	-	0.5898	1	0.5553
	Cajanus cajan (L) Millsp.	49.28 (69)	68.75 (16)	-	0.3737	1	0.7086
Kariyamangakam	*Mangifera indica* Linn.	87.39 (111)	78.46 (65)	-	-0.2932	1	0.7694
Madurai							
Othakadei	*Mangifera indica* Linn.	88.37 (86)	91.3 (23)	-	1.0746	1	0.2826
	Ceiba pentandra Linn.	25.0 (28)	57.14 (7)	-	-1.1616	1	0.2454
Valeyapatti	*Mangifera indica* Linn.	82.35 (51)	60.0 (15)	-	0.6464	1	0.5180
Virudhunagar							
Rajapalayam	*Mangifera indica* Linn.	72.31 (65)	80.0 (15)	-	1.8403	1	0.0657
Theni							
Periyakulam	*Mangifera indica* Linn.	82.35 (34)	84.21 (19)	-	-0.1246	1	0.9009
Kanyakumari							
Pechiparai	*Mangifera indica* Linn.	12.41 (162)	35.54 (447)	-	-1.5218	1	0.1281
Kundal	*Mangifera indica* Linn.	23.82 (757)	09.38 (908)	18.44 (369)	2.7836	2	0.2486
Erode	*Ceiba pentandra* Linn.	41.3 (46)	34.38 (32)	-	-0.8643	1	0.3874
Cuddalore	*Manilkara zapota* Linn.	37.5 (40)	53.13 (32)	-	1.2928	1	0.1961
Tuticorin	*Gossypium gossypioides* Ulbr.	-	21.05 (19)	-	-	-	-
Karur	*Psidium guajava* Linn.	20.59 (34)	16.67 (12)	-	-0.1852	1	0.8530

- = infested plant parts were either not infested or not available at the time of sampling. Numbers in parentheses represent the actual number of *R. iceryoides* collected per plant part during the survey.

Table 8. 3: Parasitoid complex associated with *R. iceryoides* on different host plants in the state of Tamil Nadu, India

District/Locality	Parasitoid species	Plant species	Percentage parasitism			Statistics		
			Leaves	Twigs	Fruits	Z/χ^2	*df*	*P*
Coimbatore	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	26.69 (296)	27.32 (183)	-	-0.3660	1	0.7144
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	21.28 (296)	16.39 (183)	-	-1.3229	1	0.1859
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	7.09 (296)	6.01 (183)	-	-0.2208	1	0.8253
	Parechthrodryinus excelsus Hayat	*Mangifera indica* Linn.	2.36 (296)	2.19 (183)	-	1.0849	1	0.2780
	Ericydnus paludatus Haliday	*Mangifera indica* Linn.	1.01 (296)	2.19 (183)	-	1.4187	1	0.1560
	Neoplatycerus tachikawai Subba Rao	*Mangifera indica* Linn.	2.7 (296)	3.28 (183)	-	1.0008	1	0.3169
	Agarwalencyrtus ajmerensis Fatima & Shafee	*Mangifera indica* Linn.	2.7 (296)	0	-	-1.6985	1	0.0894
	Aenasius advena Compere	*Mangifera indica* Linn.	0	4.37 (183)	-	2.2768	1	0.0228
	Praleurocerus viridis Agarwal	*Manilkara zapota* Linn.	26.11 (157)	26.92 (78)	-	1.1023	1	0.2703
	Anagyrus chryos Noyes & Hayat	*Manilkara zapota* Linn.	19.75 (157)	25.64 (78)	-	0.1225	1	0.9025
	***Coccophagus ceroplastae* Howard	*Manilkara zapota* Linn.	10.19 (157)	8.97 (78)	-	0.4229	1	0.6723
	Parechthrodryinus excelsus Hayat	*Manilkara zapota* Linn.	5.10 (157)	10.26 (78)	-	1.3703	1	0.1706
	Neoplatycerus tachikawai Subba Rao	*Manilkara zapota* Linn.	8.28 (157)	5.13 (78)	-	-0.2460	1	0.8057
	Praleurocerus viridis Agarwal	*Tectona grandis* Linn.	27.78 (54)	15.0 (78)	-	-0.4920	1	0.6228
	Anagyrus chryos Noyes & Hayat	*Tectona grandis* Linn.	20.37 (54)	25.0 (78)	-	0.1246	1	0.9009
	***Coccophagus ceroplastae* Howard	*Tectona grandis* Linn.	9.26 (54)	7.5 (78)	-	-0.6228	1	0.5334
	Parechthrodryinus excelsus Hayat	*Tectona grandis* Linn.	5.56 (54)	12.5 (78)	-	0.6708	1	0.5023
	Ericydnus paludatus Haliday	*Tectona grandis* Linn.	7.41 (54)	2.5 (78)	-	-0.5821	1	0.5605
	Praleurocerus viridis Agarwal	*Ficus benghalensis* Linn.	25.31 (162)	16.67 (54)	-	-0.2299	1	0.2187
	Anagyrus chryos Noyes & Hayat	*Ficus benghalensis* Linn.	20.99 (162)	31.48 (54)	-	1.3472	1	0.1779
	***Coccophagus ceroplastae* Howard	*Ficus benghalensis* Linn.	8.64 (162)	7.41 (54)	-	0.4229	1	0.6723
	Praleurocerus viridis Agarwal	*Psidium guajava* Linn.	28.89 (135)	25.0 (92)	-	0.4346	1	0.6639
	Anagyrus chryos Noyes & Hayat	*Psidium guajava* Linn.	16.30 (135)	20.65 (92)	-	0.1451	1	0.8847
	***Coccophagus ceroplastae* Howard	*Psidium guajava* Linn.	11.11 (135)	9.78 (92)	-	-0.5143	1	0.6070

- = infested plant parts were either not infested or not available at the time of sampling. Numbers in parentheses represent the actual number of mealybug collected per plant part during the survey. ** = Hyperparasitoids emerged from the mummified *R. iceryoides*

Table 8.3 continues. Parasitoid complex associated with *R. iceryoides* on different host plants in the state of Tamil Nadu, India

District/Locality	Parasitoid species	Plant species	Percentage parasitism			Statistics		
			Leaves	Twigs	Fruits	Z/χ^2	*df*	*P*
Salem								
Erummaiputti	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	20.27 (222)	20.42 (142)	-	-0.2861	1	0.7748
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	15.77 (222)	19.01 (142)	-	1.4486	1	0.1474
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	2.70 (222)	2.11 (142)	-	-0.0814	1	0.9351
	***Coccidoctonus terebratus* Hayat, Alam & Agarwal	*Mangifera indica* Linn.	17.12 (222)	17.61 (142)	-	-1.5000	1	0.1336
	Ericydnus paludatus Haliday	*Mangifera indica* Linn.	2.70 (222)	2.11 (142)	-	-0.0814	1	0.9351
	Leptomastidea minyas Noyes & Hayat	*Mangifera indica* Linn.	2.70 (222)	3.52 (142)	-	0.6222	1	0.5338
Dharmapuri								
Paiyur	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	15.18 (257)	21.43 (28)	-	0.0783	1	0.9388
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	24.13 (257)	28.57 (28)	-	-0.5363	1	0.5918
	Anagyrus mirzia Agarwal & Alam	*Mangifera indica* Linn.	3.89 (257)	0	-	-1.9291	1	0.0537
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	5.84 (257)	0	-	-1.6714	1	0.0016
	Praleurocerus viridis Agarwal	*Ceiba pentandra* Linn.	18.60 (43)	16.67 (12)	-	-0.4025	1	0.6873
	Anagyrus chryos Noyes & Hayat	*Ceiba pentandra* Linn.	20.93 (43)	16.67 (12)	-	-0.1246	1	0.9009
	***Coccophagus ceroplastae* Howard	*Ceiba pentandra* Linn.	6.98 (43)	0	-	-1.1739	1	0.0008
Periyapatti	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	21.14 (369)	13.91 (115)	-	-1.3577	1	0.1746
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	23.04 (369)	21.74 (115)	-	-0.0589	1	0.953
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	8.67 (369)	10.43 (115)	-	0.6505	1	0.5154
	Praleurocerus viridis Agarwal	*Cajanus cajan* (L) Millsp.	11.59 (69)	6.25 (16)	-	-0.6708	1	0.5023
	Anagyrus chryos Noyes & Hayat	*Cajanus cajan* (L) Millsp.	18.84 (69)	25.0 (16)	-	0.4229	1	0.6723
	***Coccophagus ceroplastae* Howard	*Cajanus cajan* (L) Millsp.	8.70 (69)	6.25 (16)	-	-0.9391	1	0.3477
Kariyamangakam	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	17.12 (111)	24.62 (65)	-	0.3765	1	0.7066
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	20.72 (111)	20.0 (65)	-	0.1474	1	0.8828
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	8.11 (111)	9.23 (65)	-	-0.6159	1	0.5380

- = infested plant parts were either not infested or not available at the time of sampling. Numbers in parentheses represent the actual number of mealybug collected per plant part during the survey. ** = Hyperparasitoids emerged from the mummified *R. iceryoides*

Table 8.3 continues. Parasitoid complex associated with *R. iceryoides* on different host plants in the state of Tamil Nadu, India

			Percentage parasitism			Statistics		
District/Locality	Parasitoid species	Plant species	Leaves	Twigs	Fruits	Z/χ^2	*df*	*P*
Madurai								
Othakadei	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	24.42 (86)	13.04 (23)	-	-1.0104	1	0.3123
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	24.42 (86)	21.74 (23)	-	-0.1641	1	0.8696
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	6.98 (86)	13.04 (23)	-	0.1811	1	0.8563
	Praleurocerus viridis Agarwal	*Ceiba pentandra* Linn.	10.71 (28)	42.86 (7)	-	-0.4613	1	0.0046
	Anagyrus chryos Noyes & Hayat	*Ceiba pentandra* Linn.	39.29 (28)	28.57 (7)	-	1.1983	1	0.2308
	***Coccophagus ceroplastae* Howard	*Ceiba pentandra* Linn.	10.71 (28)	0	-	1.3229	1	0.0001
Valeyapatti	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	27.45 (51)	13.33 (15)	-	1.2478	1	0.2121
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	31.37 (51)	20 (15)	-	1.5884	1	0.1122
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	9.80 (51)	13.33 (15)	-	0.0995	1	0.9207
Virudhunagar								
Rajapalayam	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	16.92 (65)	33.33 (15)	-	0.3300	1	0.0211
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	23 (65)	20 (15)	-	-0.5413	1	0.5883
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	9.23 (65)	13.33 (15)	-	0.1114	1	0.9113
	Ericydnus paludatus Haliday	*Mangifera indica* Linn.	4.62 (65)	0	-	-1.4427	1	0.0319
Theni								
Periyakulam	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	41.18 (34)	42.11 (19)	-	0.1303	1	0.8963
	Aenasius advena Compere	*Mangifera indica* Linn.	11.74 (34)	10.53 (19)	-	0.2697	1	0.7874
Kanyakumari								
Pechiparai	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	12.93 (162)	10.16 (447)	-	0.9131	1	0.3612
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	5.90 (162)	8.72 (447)	-	-0.1409	1	0.2539
	Aphycus sapporoensis Compere & Annecke	*Mangifera indica* Linn.	2.35 (162)	1.23 (447)	-	0.2311	1	0.3661
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	3.26 (162)	5.29 (447)	-	-0.6900	1	0.4902
	Leptomastidea minyas Noyes & Hayat	*Mangifera indica* Linn.	1.09 (162)	2.79 (447)	-	0.5620	1	0.5741
Kundal	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	6.95 (757)	1.92 (908)	8.28 (369)	2.7977	2	0.2469
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	4.62 (757)	3.10 (908)	4.16 (369)	4.9536	2	0.0840
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	5.14 (757)	2.59 (908)	2.13 (369)	22.7534	2	< 0.0001
	Leptomastidea minyas Noyes & Hayat	*Mangifera indica* Linn.	1.06 (757)	0.33 (908)	0	1.3729	2	0.5034
	Carabunia bicoloripes Hayat	*Mangifera indica* Linn.	0.79 (757)	0.33 (908)	0	0.7290	2	0.6946

- = infested plant parts were either not infested or not available at the time of sampling. Numbers in parentheses represent the actual number of mealybug collected per plant part during the survey. ** = Hyperparasitoids emerged from the mummified *R. iceryoides*.

Figure 8. 2: Catalogue of indigenous primary parasitoids recovered from *R. iceryoides* in the state of Tamil Nadu, India.

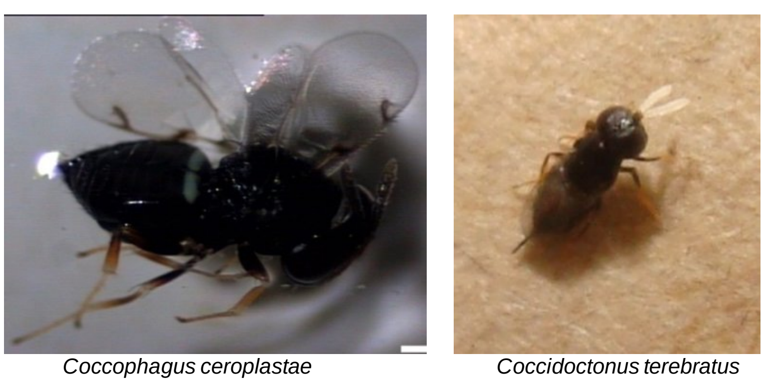

Figure 8. 3: Catalogue of hyperparasitoids recovered from mummified *R. iceryoides* in the state of Tamil Nadu, India.

8.3.4 Predators associated with *R. iceryoides* in the state of Tamil Nadu, India

In addition to the parasitoids, 10 predators from 7 families were recorded. These included, *Spalgis epius* (Westwood), (Lepidoptera: Lycaenidae), several species of coccinelids namely: *Crytolaemus montrouzieri* Mulsant, *Hyperaspis maindroni* Sicard, *Scymnus coccivora* Ayyar and *Chilocorus nigritus* Fabricius. Among these, the predaceous beetle, *C. nigritus* was the most abundant and widespread species. Brown lacewing, *Hemerobius* sp and green lacewing, *Chrysopa* sp., were also found feeding on *R. iceryoides*. Furthermore, dipteran predators from 3 different families were collected: *Leucopis* sp. (Chamaemyiidae: Diptera), *Cacoxenus perspicax* Knab (Drosophilidae: Diptera), Hover fly-larvae (Syrphidae: Diptera), implying they could be rapacious predators of *R. iceryoides*. The predaceous drosophilid, *C. perspicax* was the most abundant in this group and was commonly encountered in high density among mealybug colonies.

8.3.5 Ant species associated with *R. iceryoides* in the state of Tamil Nadu, India

Five major ant species were observed tending *R. iceryoides* in the field but their distribution was greatly varied from one location to the other: *Camponotus* (*Myrmosericus*) *rufipes* Fabricius, *Camponotus barbaricus* Emery, *Monomorium pharaonis* Linnaeus, *Crematogaster cerasi* Fitch and *Oecophylla longinoda* Latreille. The most remarkable interaction of these ant species was observed with *C. rufipes*, transporting *R. iceryoides* from mango tree top to the roots where they were nesting and actively attacking the larva of *S. epius* preying on ovipositing female of *R. iceryoides*.

8.3.6 Climatically suitable and similar regions in Southern Asia, Africa and the Americas

The projected distribution of *R. iceryoides* in Southern Asia conforms well to its known published distribution, which encompasses Indonesia, and Sri Lanka except in Bangladesh, Malaysia, Singapore and China. Apart from the above mentioned countries, new occurrence points climatically suitable for *R. iceryoides* and its natural enemies was successfully predicted by the two models in Bangkok, Cambodia, Thailand and Philippines (Figure 8.4). GARP predicted higher suitability further inland from the coastlines, while Maxent indicated suitability more restricted to isolated pockets in these parts when high threshold values are taken into account only. By definition all of the input locations derived from the distribution records had perfect match with themselves, therefore regions with highest climatic similarity values in the GARP model (Figure 8.4) corresponded perfectly with the distribution records. The climatic similarity of points in the State of Tamil Nadu decreased with distance from the input points. The rate at which the similarity decreased appeared to follow logical topo-climatic gradients.

Projecting niche models to Africa (Figure 8.5) again yielded similar predictions between the two methods, with Maxent again appearing more conservative. Both models predicted high suitability in the East African coastal regions and marginal suitability in the Equatorial rain forest belt. Invaded areas for the purpose of model validation were in East Africa, where most reported non-native mealybug populations are located (Figure 8.5). The coincidence of known records of this species in Africa with the area predicted by the projection of the native-range model was excellent, again statistically significantly more coincident than random models (χ^2 tests, all $P < 10^{-6}$).

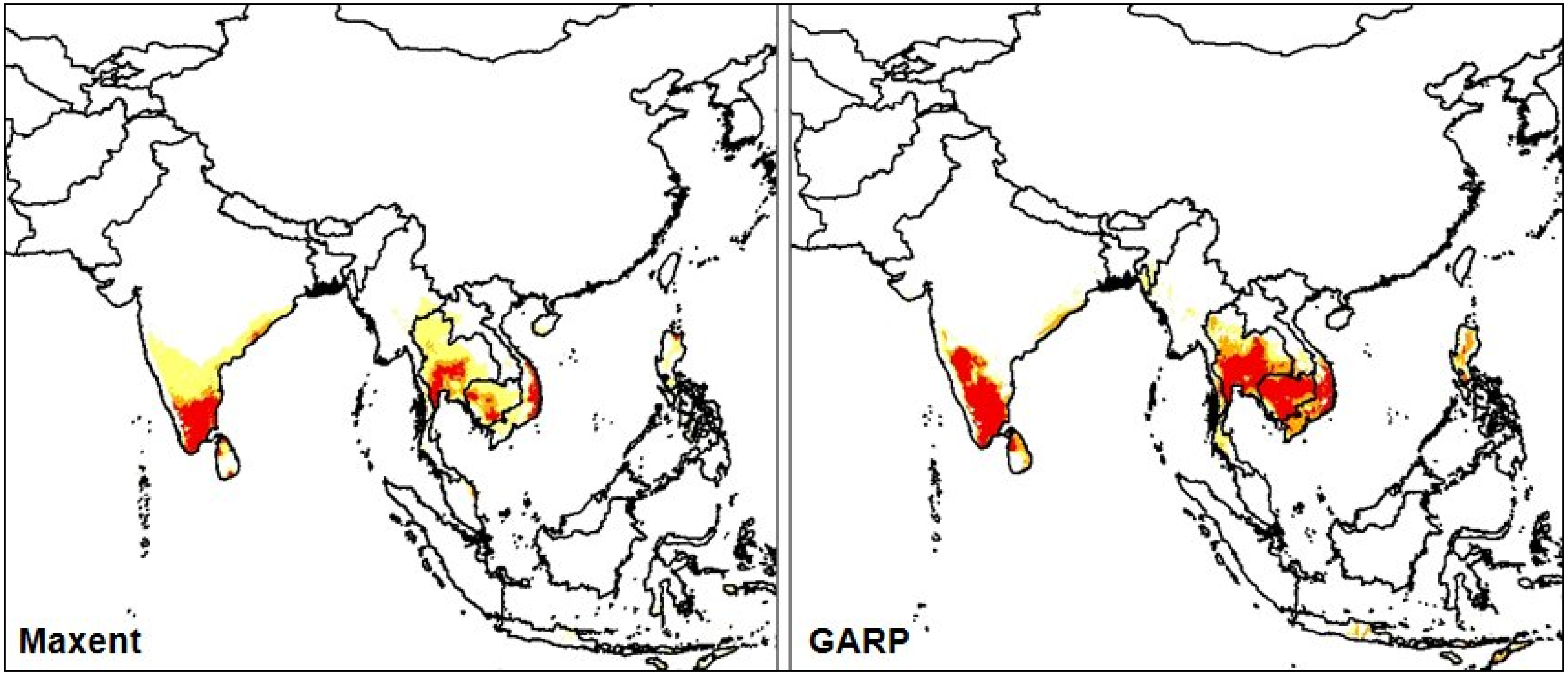

Figure 8. 4: Climatic suitability for *R. iceryoides* in its native range, Southern Asia, using genetic algorithm for rule-set prediction (GARP) and maximum entropy method (Maxent). White, predicted no suitability, as indicated by the LTPT thresholding; shades of orange indicate higher levels of climatic suitability (chosen arbitrarily), with red the highest strength for climatic suitability.

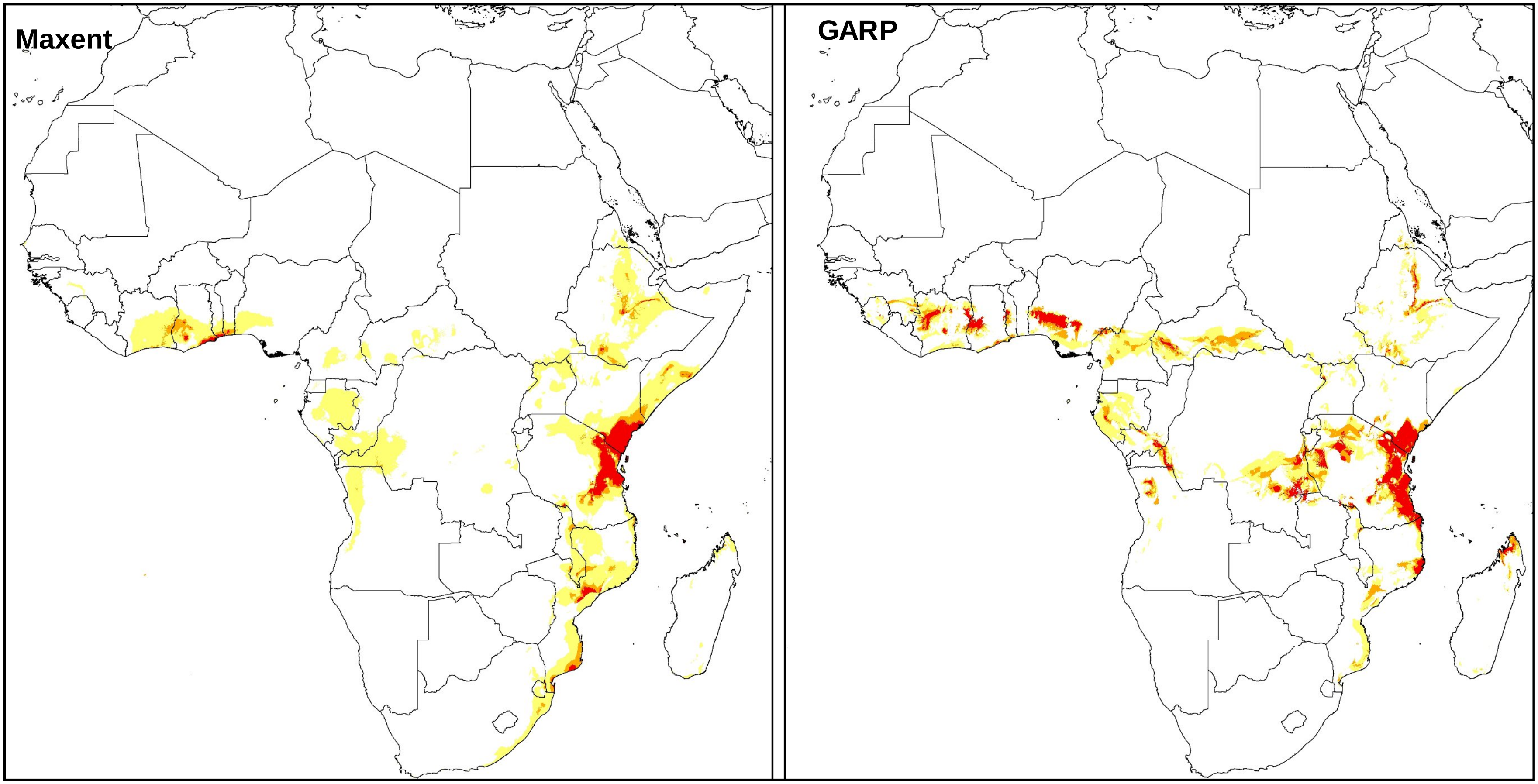

Figure 8. 5: Climatic suitability for *R. iceryoides* in its non-native range, Africa, (using India distribution records), using genetic algorithm for rule-set prediction (GARP) and maximum entropy method (Maxent). White, predicted no suitability, as indicated by the LTPT thresholding; shades of orange indicate higher levels of climatic suitability (chosen arbitrarily), with red the highest strength for climatic suitability.

The GARP model predicted higher suitability in areas farther removed from the coast, particularly in Kenya, Tanzania, Côte d'Ivoire, Ghana, Togo, Benin, Nigeria, Cameroon, Somalia and Mozambique. Also, the latitudinal limits identified by GARP predictions were broader, especially north-wards, with high suitability being predicted for much of the Tanzania, Côte d'Ivoire, Ghana, Togo, Nigeria and Cameroon; these differences were less dramatic once lower thresholds were considered in Maxent. The climatic suitability of parts of East and West Africa, and their close proximity to Central Africa underlines the threat of invasion to this area by this emerging polyphagous pest. Additionally, a large part of Mozambique also appears to be vulnerable to invasion. The regions identified above are at risk of unintentional introductions of *R. iceryoides*, and when intentionally introduced might flourish. Madagascar's proximity to the primary source of *R. iceryoides*, the Indian Subcontinent, also puts it at high risk of colonization.

Climatic suitability for *R. iceryoides* globally using India and Africa distribution records revealed that GARP predicted somewhat broader potential distributional areas in South and Central, and the Caribbean that appeared climatically similar to the regions of the state of Tamil Nadu, India and Africa, not yet colonized. For the GARP model, seven fairly large areas are indicated as being highly suitable, which covers southern and eastern Brazil, Southern Mexico, Cuba, Venezuela, Honduras, Guatemala and Haiti. Small isolated regions along the slopes of the Andes mountain range in Bolivia, Guyana and Suriname as well as in Nicaragua are also indicated as being suitable (Figure 8.6). In contrast, Maxent indicated that much of Mexico, Guatemala, Honduras, Nicaragua, Cuba, Haiti, Domanica Republic, Venezuela, Guyana, Suriname, Brazil, Paraguay and Bolivia were climatically similar to Africa and India, although much of this was marginal similarity. Only Maxent indicated that Paraguay was climatically suitable or similar to Africa and India. The GARP model indicated a broader region of climatic similarity than the Maxent in all part of the world predicted by the two models. One of the regions of highest suitability in the Maxent model is located in eastern Brazil whereas the region of highest similarity in the GARP model was found in Cuba. Northern Territory of Australia to a lesser extent was also predicted as a potential climatically suitable zone by GARP.

Non-native populations of *R. iceryoides* in Africa were used as a means of testing model predictivity regarding suitable areas for the species globally. In both cases, model predictions were considerably better than expectations under random (null) models (binomial tests, both $P <$

10^{-14}), indicating that both approaches offer significant predictivity regarding the global potential distribution of the species. Inspecting ROC plots for the two model predictions based on independent testing data on a landscape distant from that where the models were trained, it is clear that the two models are slightly similar in performance.

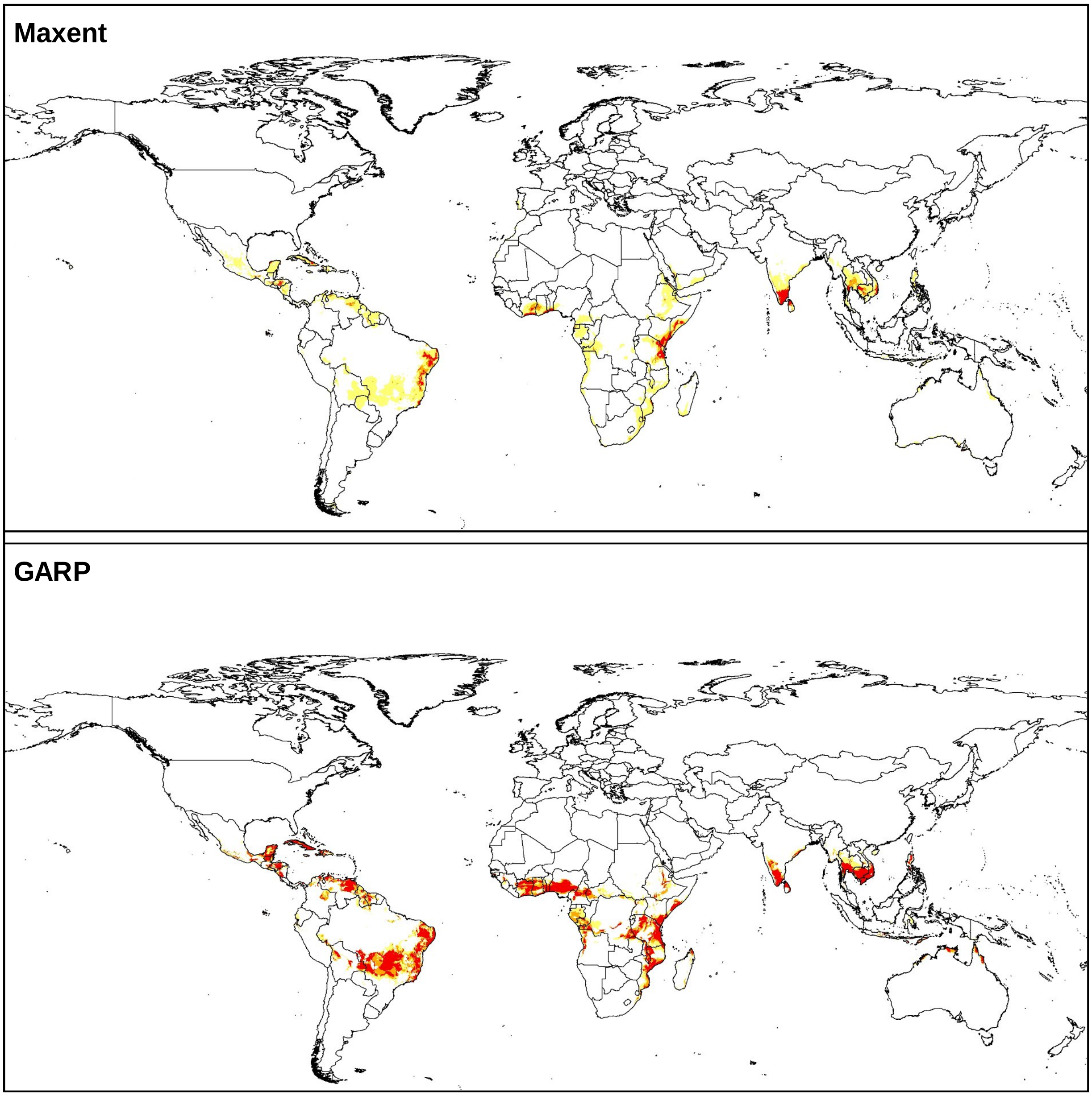

Figure 8. 6: Climatic suitability for *R. iceryoides* globally, (using India and Africa distribution records), using genetic algorithm for rule-set prediction (GARP) and maximum entropy method (Maxent). White, predicted no suitability, as indicated by the LTPT thresholding; shades of orange indicate higher levels of climatic suitability (chosen arbitrarily), with red the highest strength for climatic suitability.

8.3.7 Biological interpretation

Based on jackknife analyses for models including each variable alone, 'temperature seasonality' was the bioclimatic variable that contributed most to the ecological niche model with the highest predicted suitability for the likelihood of establishment of the parasitoids in Africa, followed by 'annual temperature range', 'annual precipitation', and 'precipitation of the coldest quarter' while the maximum temperature of the warmest month was the least important. This ecological niche model prediction does in fact appear quite similar to the distributions of the species and beyond. The areas indicated by the models contain conditions suitable for both pest and their natural enemies, because successful establishment may also be influenced by other factors, biotic and abiotic due to some geographical barriers. Maxent and GARP predictions are continuous, and within those areas suitable for the species, they further distinguish between those with a marginally (but sufficiently) strong prediction versus those with increasingly stronger predictions. This represents an important advantage for both models, and explains part of their consistently higher AUC values. Most strikingly, the models correctly indicate an expansive region of unsuitable environmental conditions for the pest and its associated natural enemies.

These parasitoid species occurs naturally in India and most of the predictions for the success of this species to become established in Kenya and Tanzania were correct. This was not surprising as there were many countries in Africa and Southern Asia that climatically matched the area-of-origin and suggests a high likelihood of establishment in these regions. Climatic modeling of potential distribution by Maxent and GARP models showed that the predicted optimal climate suitability of the parasitoids in Southern Asia and Africa (introduced home) are confined mainly to the coastal zones, where temperatures are warmer and humidity was high with the exception of desert regions where dry stress and/or hot stress might limit their establishment.

8.4 Discussion

Rastrococcus iceryoides is a primary mealybug pest of mango in India but the overall survey in the State of Tamil Nadu, India demonstrates that although *R. iceryoides* is widely distributed throughout the mango belt, they are certainly not of anything other than local importance. This is because of the existence of a complex of natural enemies capable of suppressing the mealybugs population (Tandon and Lal, 1978; Narasimham and Chako, 1988). In this study the parasitoid community was composed of eleven primary parasitoid species recovered from *R. iceryoides*, out of which seven are new records. Among these parasitoid species, *P. viridis* and *A. chryos* were clearly the most abundant and widely distributed primary parasitoids of *R. iceryoides* in the State of Tamil Nadu. Both parasitoids are highly host specific, cosmopolitan, solitary endoparasitoids of *R. iceryoides* (Narasimham and Chacko, 1988).

However, despite their important role in suppressing the populations of *R. iceryoides*, they have never been used in biological programmes (classical or inundative) against *R. iceryoides* anywhere in the world. Given the high parasitism rate of these two parasitoids across the different host plants in the field, they should be considered as candidates for biological control against *R. iceryoides* in regions other than India where they are scarce or absent in new-association strategy. *Praleurocerus viridis*, in particular has been attributed with remarkable regulation of population of *R. iceryoides* on guava resulting in complete disappearance of the pest in some part of the year in India (Mani and Krishnamoorthy, 1998b**)**. Pre-release quarantine evaluation of these parasitoids on *R. iceryoides* is now needed although it must be noted that for a parasitoid to successfully establish in a locality it must overcome a series of challenges including interspecific competition with native parasitoid species, host plant-parasitoid interaction in the invaded range among others.

The findings from the field survey reveal that these two parasitoids coexist together and also complement each other in all the localities and the host plants surveyed. Among all the host plant species sampled, the highest percent parasitism by *P. viridis* and *A. chryos* was frequently found on mango plants and occasionally on *C. pentandra, P. guajava* and *T. grandis*. Our study provides information that predicts the distribution of parasitism across host plants, which is crucial for rational conservation and augmentation of the parasitoid species. The remaining parasitoid species played a minor role and are observed to have a patchy distribution occuring in very low numbers throughout the survey. The number of new records generated from this study emphasizes the lack of attention to this pest in India because of its minor pest status. Despite the wide geographic distribution of *R. iceryoides,* this paper represents the first comprehensive study of the field-population analysis of the pest, host plant preferences and the possible role of its associated parasitoids.

The two niche modeling algorithms used in this study present a similar overall picture, although Maxent is somewhat more conservative. Comparing with the updated Koppen-Geiger climate classification (Kottek et al., 2006), most suitable areas identified by our models fall within the Equatorial climate categories (minimum temperatures (≥ 18°C)). Our result suggests that *R. iceryoides* prefers hot and humid environments with low annual precipitation, although it does not have to be continuous. This equatorial climate type should have a distinct dry period with driest-month precipitation of 0 mm and wettest-month precipitation of < 300 mm rainfall (C. M. Tanga, unpublished data). Population dynamics study for *R. iceryoides* conducted in the coast region of Tanzania for 77 weeks from June 2008 to June 2010 showed that the Coastal region of Tanzania situated in the transition zone between bimodal and unimodal rainfall belts with a distinct dry season is suitable for *R. iceryoides* survival all the year-round, although populations increase dramatically during the dry season between December to February (Tanga, unpublished data).

Mealybugs are generally known to be excellent invaders (William, 1986; Williams and Granara de Willink, 1992; Sagarra and Peterkin, 1999; Kumashiro et al., 2001; Larrain, 2002; Neuenschwander, 2003; Williams, 2004; Abbas et al., 2005; Zaka et al., 2006; Akintola and Ande, 2008; Muniappan et al., 2008; Charleston and Murray, 2010; Winotai et al., 2010; Muniappan et al., 2011) but for majority of the species, no analysis of their global invasive potential has yet been undertaken. Species distributions represent the combined effects of abiotic requirements of species, biotic interactions with other species, and limitations on dispersal (Soberon and Peterson, 2005) and ecological niche modeling provides a means of evaluating ecological factors that participate in delineating a species' geographic distribution. These analyses represent an attempt to address this question making use of computational tools to model the ecological niche of *R. iceryoides* and predict its worldwide potential distribution. The tests in three regions (Native range, Southeast Asia, Central America, South America, West Africa and East Africa) suggest that these areas are indeed climatically suitable for *R. iceryoides* populations' establishment and useful in predicting biological agent introduction. This situation contrasts with the ''pest status'' of *R. iceryoides* populations in the Central and South America (especially Mexico, Cuba, Guatemala, Nicaragua, Guyana, Suriname, Honduras, Haiti, Bolivia and Brazil) as well as in Africa (especially Cameroon, Nigeria, Benin, Togo, Ghana, Côte d'Ivoire, Central African Republic, Mozambique, Gabon, Congo, Angola, Somalia and Ethiopia), where our models show high agreement predicting potential presence. These areas predicted as potentially suitable are novel relative to known *R. iceryoides* occurrences but implies that the species is likely to expand its range further in these regions if the six barriers that a species has to overcome to become invasive can be breached by the mealybug (Richardson and van Wilgen, 2004). Given the apparently high dispersal capabilities of this species (C.M. Tanga, unpublished data), particularly in light of huge pockets of population outbreaks now developing in Tanzania and Kenya that may act as new nuclei for expansion, these areas should be considered susceptible to invasion by *R. iceryoides*. Madagascar's proximity to the primary source of *R. iceryoides*, the Indian Subcontinent, also puts it at high risk of colonization. Finally, these distributional possibilities may be in the process of shifting, given current global shifts in climates, which would in general act to broaden the species' distributional potential at the equatorial limits of its pre-change distribution.

Given the apparent rapid spread of *R. iceryoides* in Tanzania and Kenya, and its impact on local horticulture, the risk of this species being introduced, establishing and invading other regions of the world should be considered. The two models used in this study clearly indicate regions of the world that are climatically suitable for *R. iceryoides*, but this does not implies that these regions will unavoidably become invaded by the species. This is because for a species to invade in a new region, it must overcome a series of challenges (Richardson and van Wilgen, 2004; De Meyer et al., 2008). Our analyses are only able to assess one of them, which is the likelihood of the species surviving in the new region. Therefore, as this study has not explored the entire invasion challenges reported by Richardson and van Wilgen (2004) that non-native species face, these maps should not be interpreted as maps of invasion risk or likelihood of establishment. However, it is important to note that regions presenting suitable climatic conditions for the species, as indicated by the models, are more likely to be invaded than regions that have a low suitability. Most of the regions highlighted as highly suitable by the models include areas already invaded by the species, giving some confidence in the models. Several regions in the native-range of the pest, India: Andaman and Nicobar Islands, Assam, Bihar, Delhi, Gujarat, Jammu and Kashmir, Kerala, Madhya Pradesh, Uttar Pradesh and West Bengal, has been reported by Williams (1989) to harbour populations of *R. iceryoides* but were not identified to be climatically similar using the two climate matching approach. This could probably be attributed to the fact that a climate matching approach that only samples part of the distributional range of the pest has been reported to give an incomplete description of the species fundamental niche, which results in a spatial prediction that described only part of its potential range (Wharton and Kriticos, 2004; Soberon and Peterson, 2005). However, these models clearly identified Karnataka where the two parasitoids have been reported to coexist as climatically similar to Tamil Nadu (Noyes and Hayat, 1994).

In order to avoid prediction errors due to the presence of its natural enemies, wherever possible, when modeling the potential climatic suitability, use is made of distribution information from the species' exotic range, as well as its native range (Kriticos and Randall, 2001). Given that *R. iceryoides* has a much broader ecological niche in its native range, a more thorough inventory for the species in its native region, or at least detailed inspection and re-evaluation of *R. iceryoides* records from the region, might present additional information that could improve the models. Currently, however, such information is not available.

Predicting the probability of successful establishment and invasion of alien species at a broad scale, by climatic matching is a priority for the risk assessment. These models identified several areas in Africa having maximum suitability for the invasive mealybug, *R. iceryoides*, entailing a maximum risk of successful invasion. *Rastrococcus iceryoides* has already been introduced into three of these areas and in some of them invasion is ongoing. The observation stresses the importance of implementation of an early detection and eradication plan of this alien invasive species within the areas affected and showing high suitability for the pest. However, the low suitability of some areas of Africa does not mean that *R. iceryoides* can be introduced without any risk of invasion. The approach presented here can be used to focus on preventive monitoring in areas that are more at risk. Indeed, being a correlative method, these approaches does not consider directly the effects of biotic interactions that are known to be fundamentally important for the establishment and spread of introduced species or their natural enemies.

It is very useful for estimating climatically suitable areas for biocontrol introductions, for estimating the effect of climate on invasions by the exotic mealybug, *R. iceryoides* and for conservation decisions. Maxent and GARP accurately predicted when there was going to be successful establishment of the parasitoids probably because these species were overridingly influenced by climatic conditions. The accuracy of the predictions also depended on the preciseness of the physiological parameters derived for the species. This emphasizes the importance of determining the exact original distribution of the species to accurately derive the parameters and iterate the models until the highest values are obtained. Both models operate on a wider spatial and temporal climatic scale with less emphasis on microclimatic factors such as amount of solar radiation, degree of cloudiness or frequency of wind, all of which are important to a small ectotherm (Unwin and Corbet, 1991). Although climate may appear similar between two sites, other factors such as distance from the sea and elevation can also influence local fluctuations in temperatures and rainfall during the year. This could explain the disparity in the models in equally predicting the potential establishment of the pest and parasitoids in different regions of Africa and the world at large. The models for the invasive pest did correctly predict the likelihood of establishment of the parasitoid species in new geographical areas of Africa and suggested that overall climate might be the sole determinant of establishment, although other factors can play a major role.

Because the Maxent and GARP models were fitted primarily to the currently known native distribution, the model probably represents the realized niche of *R. iceryoides* and, therefore, it is likely to be partially conservative because of the presence of biotic constraints namly competitors, predators and natural enemies in the native range (Davis et al., 1998). As noted by Brown et al. (1996), where biotic factors are constraining ranges, they are most likely to manifest themselves in those regions of the potential range that are relatively warm and wet, and where environmental resources such as heat and moisture are not limiting. Hence, as the warm and wet range limits for *R. iceryoides* are not encountered in Kenya and Tanzania, the model can be considered to be reasonably reliable, and potential climatic range expansion due to biotic release is unlikely. Therefore, for critical decision making, it is prudent to consider the sensitivity of the affected system to be the probable full range of expected climatic suitability.

CHAPTER NINE

General discussion, Conclusion and Recommendations

9.1 General discusion and conclusion

Mealybugs (Hemiptera: Pseudococcidae) are important group of phytophagous insects that cause significant damage on a variety of horticultural crops worldwide. In Africa, *R. invadens* and *R. iceryoides* are regarded as two important exotic mealybug species native to Southern Asia that commonly colonize mango, *M. indica*. The former devastated mango production in West and Central Africa, but was brought under biological control through the introduction of the exotic parasitoid *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae) from India. Owing to the devastating nature and the socioeconomic impact that *R. invadens* had on the livelihood of farmer in West and Central Africa, it has been the subject of many studies and a considerable amount of information was gathered and documented with regards the pest host range, geographical distribution and it natural enemies. *Rastrococcus iceryoides* on the other hand is so far restricted to East Africa (mainly Tanzania and coastal Kenya) and northern Malawi where it has remained as a major pest of *M. indica*.

Due to the novelty status of *R. iceryoides* compared to *R. invadens* there had been little known about the biology and ecology of this pest and its associated natural enemies that would inform development and implementation of any management strategies against this pest in the invaded areas in Africa. Consequently, this study was initiated to study the bio-ecology of *R. iceryoides*, assess the role of indigenous natural enemies in suppressing the pest; and explore for effective co-evolved natural enemies in its aboriginal home for introduction and release in target African countries. The effect of climatic factors on the seasonal and annual dynamics of the pest and its natural enemies in Tanzania was also investigated. Finally, laboratory studies were carried out to unravel the effect of host plants on the development and reproduction of *R. iceryoides* and *A. pseudococci* as well as the interference of *Oecophylla longinoda* in the biological control of *R. iceryoides* by *A. pseudococci*.

Chapter 3 describes the distribution, host-plant relationship and natural enemies survey of *R. iceryoides* in Kenya and Tanzania. The results showed that *R. iceryoides* is widely distributed across the coastal belt with heavy infestation levels extending up to 145 km inland in Kenya and 851 km in Tanzania. In Kenya, the high level of *R. iceryoides* infestations in Matuga is particularly disturbing because this locality represents one of the key mango production areas in the country while in Tanzania, the high level of attack on mango in Kinondoni and Mkuranga demands urgent management attention given the ongoing expansion of the horticulture industry and particularly mango in the region. *Rastrococcus iceryoides* was recorded from 29 plants species including cultivated and wild host plants from 16 families, 21 of which are new records for Kenya and Tanzania. The wild host plants recorded during the survey harbouring this invasive pest occur throughout the year and evidently ensured that sufficient reproductive bases existed for *R. iceryoides* as a persistent source of spread of the mealybug during the off-season when cultivated hosts were not adequately suitable for attack. The wide range of plant families attacked by *R. iceryoides* strongly suggests that this pest is an emerging polyphagous species capable of surviving in a wide range of host and can jeopardize the lucreative export of these crops from this region. There was evidence of altitudinal limits of distribution of *R. iceryoides* in both countries with indications that it is better adapted to low and mid altitudes, which exactly match its distributional range in its native home of India. Although the precise date of introduction of *R. iceryoides* to both Kenya and Tanzania is unknown, it is highly probable that current distribution and spread of the mango mealybug populations is assisted by fruits and plant materials transported across the region in commercial and private vehicles as is the case with the introduction of *R. invadens* into West and Central Africa. Although, six primary parasitoid species were recovered from *R. iceryoides* with some representing new associations only *A. pseudococci* showed promising performance (~20% parasitism). This study provides information that predicts the distribution of parasitism across host plants, which is very crucial for rational conservation and augmentation of the parasitoid. Therefore, conservation of this parasitoid (through habitat management), and its augmentation with periodical releases of laboratory reared wasps, will enhance the effectiveness of this parasitoid in suppressing the population of *R. iceryoides* in areas with low infestations.

In laboratory host preference studies carried out in chapter 4, results showed that although the six host plant species tested supported the development of *R. iceryoides*, they differed significantly in their suitability. *Mangifera indica*, *C. moschata*, *P. aculeata* and *C. cajan* were the most preferred host plants in view of improving laboratory mass rearing of this pest. The suitability of host plant species described in this study should help in the making of informed decisions regarding the invasion risk associated with the insect in countries where *R. iceryoides* has not invaded. Base on these findings, it is likely that more important host plants will be shown to support development of *R. iceryoides* as further studies are carried out. It is mostly likely that this information will become increasingly important as *R. iceryoides* continues to colonize new geographical areas. In addition to helping predict the population growth potentials, the life table findings from the different host plants in this study have practical implications to more efficient and effective production of the mealybugs for parasitoid mass rearing and releases.

Chaper 5 describes the effect of five host plant species on host acceptability for oviposition (as measured by % parasitized nymphs) and suitability (as measured by day to mummification, percent mummified host, percent parasitoid adult eclosion, sex ratio and pre-maginal developmental time) for immature development of *A. pseudococci***.** Effect host plant on fitness trait (parasitoid size, egg load and longevity) and life table parameters was also assessed. Although *A. pseudococci* accepted mealybugs regardless of the host plant species on which they were reared and exposed, the level of acceptability varied significantly, with the highest and lowest percent parasitized nymphs on butternut and weeping fig respectively. Host suitability was also strongly affected by the host plant and largely mirrored host acceptability for all the parameters evaluated. To judge from the R_o and r_m values, mass rearing would be suitable on the four most optimal host plants (*M. indica*, *C. moschata*, *P. aculeata* and *C. cajan*). These observations provide important information for future management of the mealybug. On non-crop host plants such as *P. aculeata*, targeted management methods including parasitoid conservation and augmentation on this host should result in the build-up of the parasitoid populations ahead of the mango fruiting season before heavy infestations on the mango fruits start. Overall, the information provided in this investigation should be essential in understanding the dynamics of the parasitoid on different host plants and form vital part of an integrated management plan that allows for targeted suppression of the mealybug in East Africa.

Chapter 6 revealed that *O. longinoda* showed aggressive behaviour toward *A. pseudococci,* which greatly affected the foraging activities and significantly reduced the oviposition success of *A. pseudococci*. Worker ants were also observed to remove mummified mealybugs as food source, which resulted in significantly reduced percentage of adult parasitoid eclosion. Although, *A. pseudococci* manifested some behavioural responses to escape ant aggression, they were significantly attacked and killed by worker ants. Therefore, mass release programmes of *A. pseudococci* to control low mealybug infestation in ant infested mango orchards via inundative releases should be done with considerable caution as this may not be effective depending on the ant species present. These results are important to growers who should be aware of the species of ants foraging in their orchards.

The studies on the seasonal and annual abundance of *R. iceryoides* carried out in chapter 7, indicates that the activities of *R. iceryoides* were governed by temperature, relative humidity and rainfall. The results indicated that the population dynamics of *R. iceryoides* followed an annual cycle which is synchronized with the mango fruiting season, with a peak incidence occurring during the dry season (December-February) on all plant parts at both sites. Mealybug incidence was low throughout the rainy season (March to October) and this prolonged period of low infestation, thus provide a wider window of opportunity for management. Both predators and parasitoids demonstrated the highest level of activities between December and February for each year. The incidence of the predators and parasitoids were observed to increase correspondingly to that of *R. iceryoides* infestation, which clearly illustrates the enhance ability of the parasitoids and predator populations to track the host's population. However, they were not adequate to affect the pest population probably, because of the rapid developmental time, high survival rates, enormous reproductive capacity, and lack of early detection and control of the mealybug population.

The survey carried out in chapter 8 revealed that *R. iceryoides* is a primary mealybug pest of mango in the State of Tamil Nadu, India. Although, *R. iceryoides* is widely distributed throughout the mango belt, they are certainly not of anything other than local importance. This is because of the existence of a complex of natural enemies capable of suppressing the mealybugs population. This survey reported a total of eleven primary parasitoid species, out of which seven are new records. Among these parasitoid species, *P. viridis* and *A. chryos* were clearly the most abundant and widely distributed species. Both parasitoids are highly host specific, cosmopolitan, solitary endoparasitoids of *R. iceryoides*. However, despite their important role in suppressing the populations of *R. iceryoides*, they have never been used in biological programs (classical or inundative) against *R. iceryoides* anywhere in the world. Given the high parasitism rate of these two parasitoids across the different host plant species in the field, they should be considered as candidates for biological control against *R. iceryoides* in regions other than India where they are scarce or absent in new-association strategy. Priority areas identified by the two ecological niche models, GARP and Maxent for further search for natural enemies in Southern Asia were Sri

Lanka, Bangkok, Cambodia, Thailand, Philippines, and to a lesser extend Northern Territory of Australia. In India, additional priority areas determined for further exploration of natural enemies of *R. iceryoides* included three additional states: Karnataka, Orissa and Andhra Pradesh. The potential worldwide distributional range presented by the two models is indicative of the likely magnitudes of the expected changes in the potential range of *R. iceryoides* in response to climate suitability, highlighting areas at high risk. The extensive areas of potential mango production reported in Central and South America identified by the models has not yet invaded by this invasive pest. This suggests that proper policies should be formulated and implemented in the different areas to limit the expansion of *R. iceryoides* and for early detection of the pest. In Africa, the potential distributional range described by the models are of interest to improve the communication with the general public, policymakers and other stakeholders, who wish to understand the potential spatial extent of the invasion so that the impacts can be gauged and appropriate ameliorative measures, can be considered prior to the invasion reaching valuable mango production countries.

9.2 Recommendations for application and future study

1) Host range of invasive phytophagous insects is a dynamic phenomenon particularly as a result of climate change. It is very likely that the host plant list presented here may not be conclusive data for *R. iceryoides* and may change over time. Further survey activity is therefore strongly recommended.
2) Areas without or with low pest prevalence identified in this study might benefit from a simple community-based quarantine system such as the restriction of uncontrolled movements of plant materials. So material from altitudes outside this range may offer potential for *R. iceryoides*-free source of planting material, especially if the production in these areas deploys an integrated approach to further minimize infestation risk. These findings are also likely to be of great significance in other regions of Africa, in case this pest is accidentally introduced into new regions where their natural enemies are absent as they may become particularly injurious.
3) Although several indigenous parasitoids have been found attacking the pest in Kenya and Tanzania, they are unable to keep the pest populations below economically damaging levels. Therefore, as chemical and mechanical measures have proved inefficient,

biological control remains the most efficient and cost-effective option, and would require additional species of efficient coevolved natural enemies from the aboriginal home of *R. iceryoides*, India where it is rarely a pest and under excellent natural control to solve this problem in Africa.

4) Host plant species played a significant role in influencing the life history parameters of *R. iceryoides* and the parasitoid. These differences may be attributed to nutritive factors; allelochemical compounds and physical differences in stem structures, although none of these factors involved in the variation of plant suitability was investigated.
5) This study therefore, concludes that *R. iceryoides* can adapt to the transferred host plant gradually, based on an initial adverse effect on development and reproduction during a few generations after shifting host plants. It is most likely that this information will become increasingly important as *R. iceryoides* continues to colonize new geographical areas in the African continent.
6) The findings from the tritrophic interactions between host plants, *R. iceryoides* and *A. pseudococci* demonstrate that the performance of *A. pseudococci* varied significantly on the various host plants. Therefore, for any successful biological control of the mealybugs by *A. pseudococci*, the host plant species should be taken into consideration as an important factor in the success of the biocontrol agent.
7) Disturbance by *O. longinoda* greatly affected the foraging activities and significantly reduced the oviposition success of *A. pseudococci*. Collectively, these data suggest *O. longinoda* could have a detrimental effect on populations of *A. pseudococci* in mango orchards. Therefore, ant populations should be suppressed or controlled prior to release of parasitoids to suppress populations of ant-tended Hemiptera in mango orchards.
8) The studies on the seasonal and annual abundance of *R. iceryoides* in relation to weather factors demonstrated that reproductive activities of *R. iceryoides* is continuous and its occurrence throughout the year has serious implications in their management. However, given that at lower temperatures during the southwest monsoon (June – October), populations of *R. iceryoides* declined sharply it thus provides a wider window of opportunity for management. Therefore, control programs can take advantage of the fact that the development of *R. iceryoides* is dependent on the prevailing weather conditions.

9) The two parasitoid species (i.e., *P. viridis* and *A. chryos*) identified during the foreign exploratory survey in the native home of the pest in India, were found to be very effective against the pest but have never be introduced in Africa for biological control of *R. iceryoides*. As growers await approval for possible importation of these important biocontrol agents, there is an urgent need to continue doing research on the development of *A. pseudococci* for possible suppression programs in low infestation areas in both countries.
10) Because *R. iceryoides* apparently occurs in low abundance in its native range of India, it indicates that the pest is under biological control, making the insect an excellent candidate for classical biological control in Kenya and Tanzania. However, further laboratory bioassays are necessary to test the efficacy of the dominant parasitoid species to fully incorporate them into IPM programs as prime candidate for releases.
11) The seasonal/annual population studies provide a baseline data for development of geospatial models for predicting areas where *R. iceryoides* can potentially establish. There is therefore need for the development of an early warning system especially for regions where the pest has not invaded.
12) Awareness campaigns and education of the growers about the importance of mealybugs in general and availability of management practices will be crucial for dealing with all the mealybug pests. Farmer field days and field demonstration of activities of available IPM packages should form an integral part of the mealybug pest management programs.
13) Presently management effort by mango growers in Kenya and Tanzania concentrates on the cultural methods, mainly cutting and burning of heavily infested plant materials because of reluctance of the continuous use of pesticides. As a matter of fact this practice must be revised because it is actually detrimental to predators' larvae and the parasitoid developing inside host body that died out of burning and if the infested plants are buried in the soil the parasitized mealybugs also get crushed.