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ORIGINAL ARTICLE

Classical biological control program for the mealybug *Oracella acuta* in Guangdong Province, China

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Abstract The mealybug *Oracella acuta*, native to the southeastern US, was accidentally introduced into slash pine plantations in Guangdong Province in China in 1988. A classical biological control program was initiated in 1995, and the parasitoids *Allotropa oracellae*, *Acerophaus coccois*, and *Zarhopalus debarri* were imported from the US. A total of 19 972 parasitized mealybugs were shipped to China from 1996–2004, from which 15 430 wasps emerged, 12 933 of which were the three target species. Efforts to establish a mass-rearing program for the parasitoids in China failed. Five field release sites were established, and 6 020 parasitoids were released. Only 118 individuals of the three imported species were collected during establishment checks, although several wasps were collected 1–2 years after the last parasitoid release. Over 2 000 *Anagyrus dactylopii*, a cosmopolitan parasitoid, emerged from the parasitized mealybugs collected, a majority from the Taishan area near the site of the original introduction of *O. acuta*. To date the imported parasitoids have failed to establish, and natural enemies have not noticeably reduced mealybug populations.

Key words *Acerophagus coccois*, *Anagyrus dactylopii*, *Allotropa oracellae*, classical biological control, *Oracella acuta*, *Zarhopalus debarri*

Introduction

The mealybug *Oracella acuta* (Lobdell) (Pseudococcidae: Hemiptera) is considered native to southeastern US, ranging from Maryland south to Florida and westward to Texas. Its primary hosts are loblolly (*Pinus taeda* L.), slash (*P. elliottii* Englm.), Virginia (*P. virginiana* Mill.), shortleaf (*P. echinata* Mill.), and longleaf pines (*P. palustris* Mill.) (Johnson & Lyon, 1988; Clarke *et al*., 1990). *Oracella acuta* has four and sometimes five generations per year in the southern US, although fewer generations are likely in the northern regions of its range.

The mealybugs overwinter as crawlers (first instar larvae) deep within the needle fascicles or under old resin cells produced by females of the previous generation (Clarke *et al*., 1990). Some crawlers also may overwinter under bark scales (Johnson & Lyon, 1988). Crawlers settle on the new growth when new shoots begin to expand in the spring. The shoot tip is the preferred settling site, but crawlers also settle on the inner base of needle fascicles along the entire length of the new growth (Clarke *et al*., 1990). Cones and needles also may be infested when population levels are high. The pink females settle on the shoots and construct characteristic white resin cells beneath which they feed, mature, and oviposit. Females produce honeydew, and sooty mold soon covers the twigs and foliage. Males are smaller than females and develop on the shoots.

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Males from the first generation are usually apterous, while subsequent generations are alate. Wind dispersal of the crawlers is the primary means of population spread.

Populations in the US usually are kept at low levels by a suite of natural enemies, and noticeable populations only occur when this natural enemy complex is disrupted by insecticide applications or similar disturbances (Clarke *et al*., 1990, 1992). Three species of parasitoids appear responsible for regulating populations of the mealybug in the US: the platygastrid *Allotropa oracellae* Masner, and the encyrtids *Acerophagus coccois* E. Smith and *Zarhopalus debarri* Sun (Clarke *et al*., 1990; Sun *et al*., 2004a). The latter is a solitary endoparasitoid, while the other two are gregarious endoparasitoids (Dorn *et al*., 2001; Sun *et al*., 2004b). *Allotropa oracellae* and *Z. debarri* are host-specific on *O. acuta*, while *A. coccois* has multiple mealybug hosts. An unknown signiphorid species of *Chartocerus*, a probable hyperparasitoid, also was recovered. The parasitoids can quickly reduce population outbreaks, and in the absence of insecticide applications, parasitism rates can range from 50% to over 90% (Clarke *et al*., 1990; Sun *et al*., 2004a). Predators such as lacewing larvae and two species of cecidomyiid larvae feed on the mealybugs when population levels are high.

Oracella acuta was accidentally introduced into a slash pine seed orchard near Taishan in the Guangdong Province, China in 1988, although infestations were not detected until 1990. Slash and loblolly pine had been planted extensively throughout Guangdong Province and other subtropical regions of southeast China (Huo & Hartzler, 1995). Heavy infestations soon developed in slash pine plantations near the seed orchard, and these infestations began to spread rapidly throughout the Province. Early efforts to contain and control the infestation by insecticide applications and tree removal failed (Sun *et al*., 1996). The coccinellid *Cryptolaemus montrouzieri* Mulsant and a platygastrid, *Allotropa* sp., were released in the infested area, but did not suppress the outbreak (Tang *et al*., 1995). In 1995, the infestation edge expanded by 63 km to cover over 200 000 ha. Scattered Masson pines (*P. massoniana* Lambert) within the slash pine plantations also were infested. The infestation spread into Guangxi Province in 1999, threatening plantations of loblolly and Caribbean pine (*P. caribaea*). In 2008, the infestation area reached 284 000 ha (H.B. Yu, unpublished data). Song and Pan (1994) estimated the potential range of *O. acuta* in China at over 1.5 million ha across 12 provinces (Fig. 1).

There were four or five generations per year in the current range in China (Xu *et al*., 2002; Zhou *et al*., 2003). Population numbers peaked in the summer, dropped sharply in August, then increased slightly in November and December. High temperatures, high humidity, and increased rainfall were proposed as factors in the population declines. In newly infested areas, the mealybug density per shoot was highest in the second year, declining, then leveling off in subsequent years. In Guangdong Province, *O. acuta* infestations reduced tree growth by 25%–33% (Ren *et al*., 2000). Although tree mortality was rare, virtually every pine within the expanding range of *O. acuta* was infested.

Native Masson pine forests in China had been severely impacted by introductions of the pine needle scale *Hemiberlesia pitysophila* Takagi and the pinewood nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (Wilson, 1993). Many areas affected by these two pests and replanted with slash pines were infested by *O. acuta*. The loss in yield of these plantations could lead to another severe reduction in wood supply for the region. Given the effectiveness of the parasitoids in the US and the failure of control measures in China, a classical biological program offered the best potential to greatly reduce the impact of *O. acuta* in China. Classical biological control programs against other mealybugs had been successful. Introductions of the encyrtids *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam, and Agarwal in Puerto Rico and the lower desert region of California reduced the spread and infestation levels of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Michaud & Evans, 2000; Roltsch *et al*., 2006). Bokonon-Ganta *et al*. (2002) reported that the importation of two encyrtids to control mango mealybug, *Rastrococcus invadens* Williams in Benin yielded significant economic benefits. The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, was reduced to minor pest status in parts of Africa after releases of the encyrtid *Apoanagyrus lopezi* De Santis (Neuenschwander, 2001).

A cooperative biological control program for *O. acuta* between the US and China was initiated in 1995 (Sun *et al*., 1996). The goal of the program was to import parasitoids from the US to reduce populations of *O. acuta* in China. *Allotropa oracellae* outcompeted *Z. debarri* in caged experiments, appeared very active in searching for hosts, and was also the most prevalent parasitoid when *O. acuta* populations were low (Clarke *et al*., 1990; Sun *et al*., 2004b). However, *A. coccois* or *Z. debarri* often were the predominant parasitoid species collected from *O. acuta* infestations. Therefore, all three species were selected for importation. The objective of this paper is to document the rearing, importation, release and establishment of the parasitoids from 1996 to 2004.

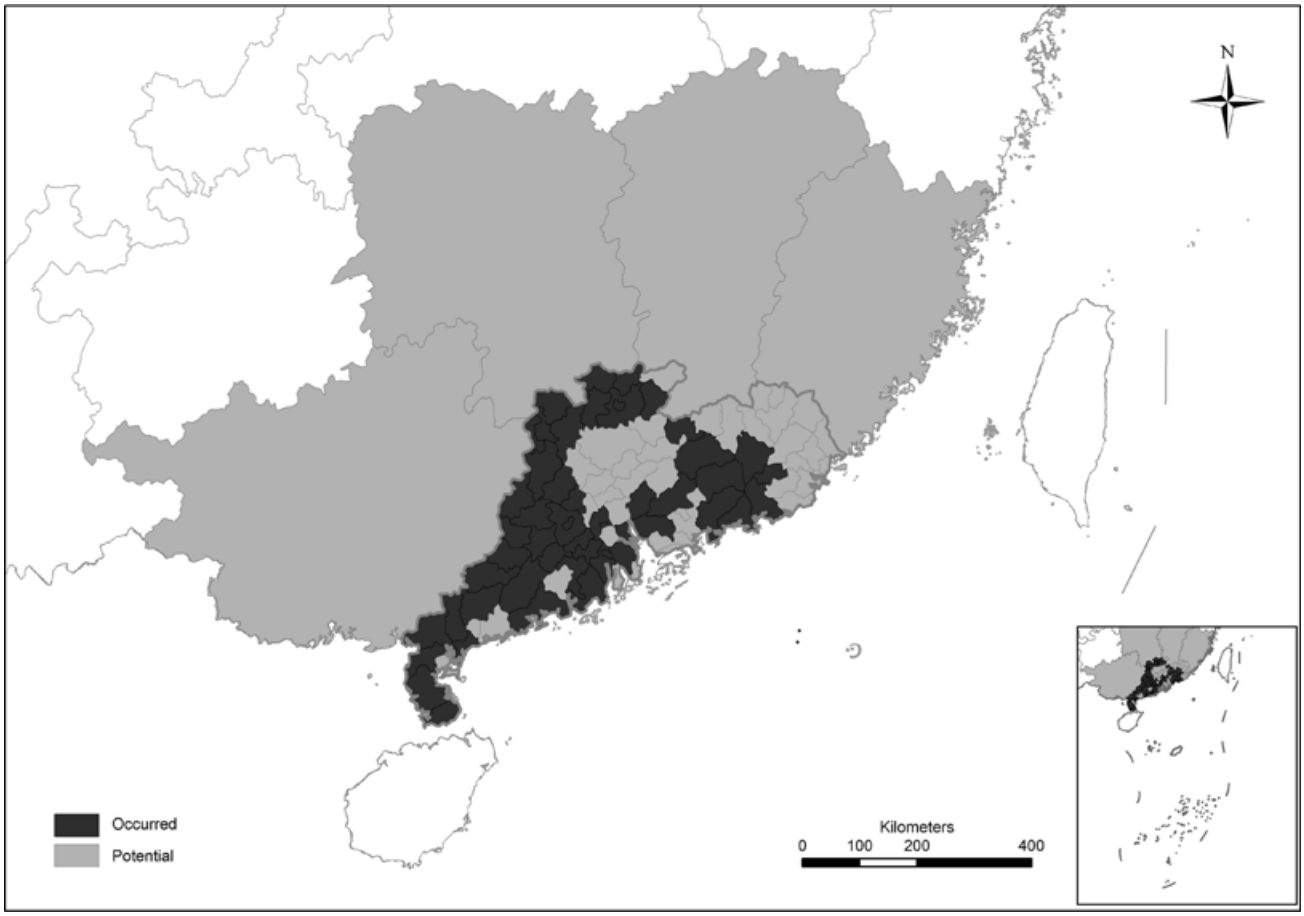

Fig. 1 Occurrence of *Oracella acuta* in 2004 and its potential distribution in China. [GS (2009)1375]

Materials and methods

Oracella collections and parasitoid rearing in the US

From 1995–2004, field collections of *O. acuta* were made routinely from infested pine seed orchards or plantations in South Carolina, Georgia, Florida, Alabama, Mississippi and Texas. Infested shoots were collected and examined under a microscope for evidence of parasitism. Parasitized mealybugs were mummified and no longer pink, turning yellow or brown. Females with a solitary parasitoid became capsule-shaped, while mummies containing multiple parasitoids had irregular shapes. If parasitism was prevalent at a site, additional infested shoots were collected and the parasitized mealybugs extracted and placed in gelatin capsules. The mummies were either shipped to China or the emerging parasitoids utilized for mass-rearing.

Mass-rearing of parasitoids was conducted in Athens, GA, US from 1995–1997 on 2–3-year-old potted loblolly pines in an enclosed section of a greenhouse. Field collections of *O. acuta* crawlers were released onto the pines to establish greenhouse populations for propagation of parasitoids. Once viable *O. acuta* populations were established on the pines, they were moved to another enclosed section of the greenhouse. Branches or entire seedlings were caged using fine mesh cloth. Parasitoids emerging in gelatin capsules were separated by species and placed in Petri dishes (15 × 100 mm). Parasitoids were supplied with fresh water and a food supplement (Eliminade^{TM}, Entopath Inc., Easton, PA, US). After 1–2 days the parasitoids were released into the cages to colonize the mealybug populations. The pines were inspected every 3 days and parasitized mealybugs were collected and placed in gelatin capsules. The mummies were shipped to China or the parasitoids were allowed to emerge and were used to

Table 1 Shipments of parasitized *Oracella acuta* females from the US to Guangdong Province, China, and numbers of parasitoids emerging.

Year	Number of shipments	Mummies	Percent† emergence	Emerging parasitoids						
				Acerophagus coccois		*Zarhopalus debarri*		*Allotropa oracellae*		Others‡
				Females	Males	Females	Males	Females	Males	
1996	6	1 174	31.3	44	41	102	73	15	19	73
1997	14	8 908	41.7	33	11	1 050	426	791	687	953
1998	8	2 103	64.1	1 318	1 064	211	160	6	35	81
1999	4	1 502	64.0	543	364	358	217	1	4	85
2000	5	1 621	61.3	80	56	199	122	0	0	634
2001	2	870	36.6	41	27	0	1	147	65	159
2002	3	360	57.8	178	125	14	10	98	72	16
2003	5	2 286	62.9	1 991	1 101	11	2	90	79	179
2004	2	1 148	59.7	164	106	187	103	189	102	277
Total	49	19 972		4 392	2 895	2 132	1 114	1 337	1 063	2 457

†Percent of mummies with at least one emerged parasitoid or hyperparasitoid.

‡Includes the hyperparasitoids *Chartocerus* sp., *Procheiloneurus* sp., *Coccidoctonus* sp., and unknown wasps.

maintain the rearing colony. The greenhouse was supplied with fresh potted pines as needed.

Parasitoid shipments

From 1996–2003, 49 shipments of parasitized *O. acuta* were made to the Forest Pest Control Station in Guangzhou (Table 1). To control the potential introduction of other pests or hyperparasitoids, infested shoots were not shipped, only mummies in gelatin capsules. Increased security and regulations enacted in late 2001 resulted in delays of up to 2 weeks for the shipments to clear customs, leading to increased parasitoid mortality. Subsequently, shipping of parasitoids was arranged through a freight shipping company, and in 2003 the shipping proceeded with a delay of only 2–3 days.

Parasitoid mass-rearing in Guangzhou

A mass-rearing facility for propagation of parasitoids was established in a greenhouse in Guangzhou, China by the Forest Pest Control Station in 1995. Initial rearing was conducted in 10 × 10 m rooms that contained approximately 30 potted loblolly pines infested by *O. acuta*. A new rearing facility was built in 2002. This new facility contained approximately 70 potted loblolly and slash pines for mass-rearing of the parasitoids.

Releases in the mass-rearing facility were made dependent on the availability of parasitoids from shipments or rearing (Table 2). Parasitoids emerging in the gelatin capsules were identified and separated by species. Known or suspected hyperparasitoids were destroyed. Initially individuals of the three target species were enclosed briefly in glass vials. The vials were taped to the stem of the infested pines in the rearing facility and the vial lid removed. One parasitoid species was released per rearing room. The rearing rooms contained water and Eliminade™ for the parasitoids. In 1998 and thereafter, the parasitoids were first kept in Petri dishes with food and water for 1–2 days. The parasitoids were then released onto caged branches or trees in the rearing facility. The cages were removed 3 weeks later. The pines in the rearing facility were replaced with fresh infested pines as needed.

The mealybugs in the rearing facility were periodically checked for signs of parasitism. If parasitism was evident, the shoots were clipped, examined under a microscope, and all mummified *O. acuta* placed in gelatin capsules. As some parasitized mealybugs had not yet displayed evidence of mummification, the shoots were then enclosed in cardboard boxes to allow parasitoids to develop and emerge. The boxes varied in size, but generally were <0.2 m^3. Each box had a 1.3 × 6.5 cm glass vial inserted into the top. The boxes were placed under a light source so emerging parasitoids were attracted to the light and congregated in the vial. Parasitoids collected in the vials or capsules were identified and utilized for either mass-rearing or for field releases.

Table 2 Releases of parasitoids of *O. acuta* in the rearing facility in Guangzhou, China.

Year	Number of releases	Trees or caged branches used	*Acerophagus coccois*		*Zarhopalus debarri*		*Allotropa oracellae*	
			Females	Males	Females	Males	Females	Males
1996	7	8	35	32	71	44	11	8
1997	52	100	15	11	269	184	152	155
1998	16	58	173	236	29	34	3	7
1999	9	29	141	96	25	7	0	0
2000	36	56	25	13	86	164	0	0
2001	16	19	32	21	0	0	136	60
2002	14	26	130	57	9	7	33	26
2003	5	68	851	534	12	3	84	62
2004	19	36	158	105	182	102	126	74
Total	174	400	1 560	1 105	683	545	545	392

Field releases

Field releases were made at five slash pine sites in Guangdong Province (Fig. 2). The Taishan site was near the seed orchard where *O. acuta* was first introduced in 1988. All three parasitoid species were released at this site (Table 3). Each species was released into a separate slash pine plantation, although the release sites were <1 km apart. The trees in these plantations were approximately 25 years old, with a diameter of 15–21 cm.

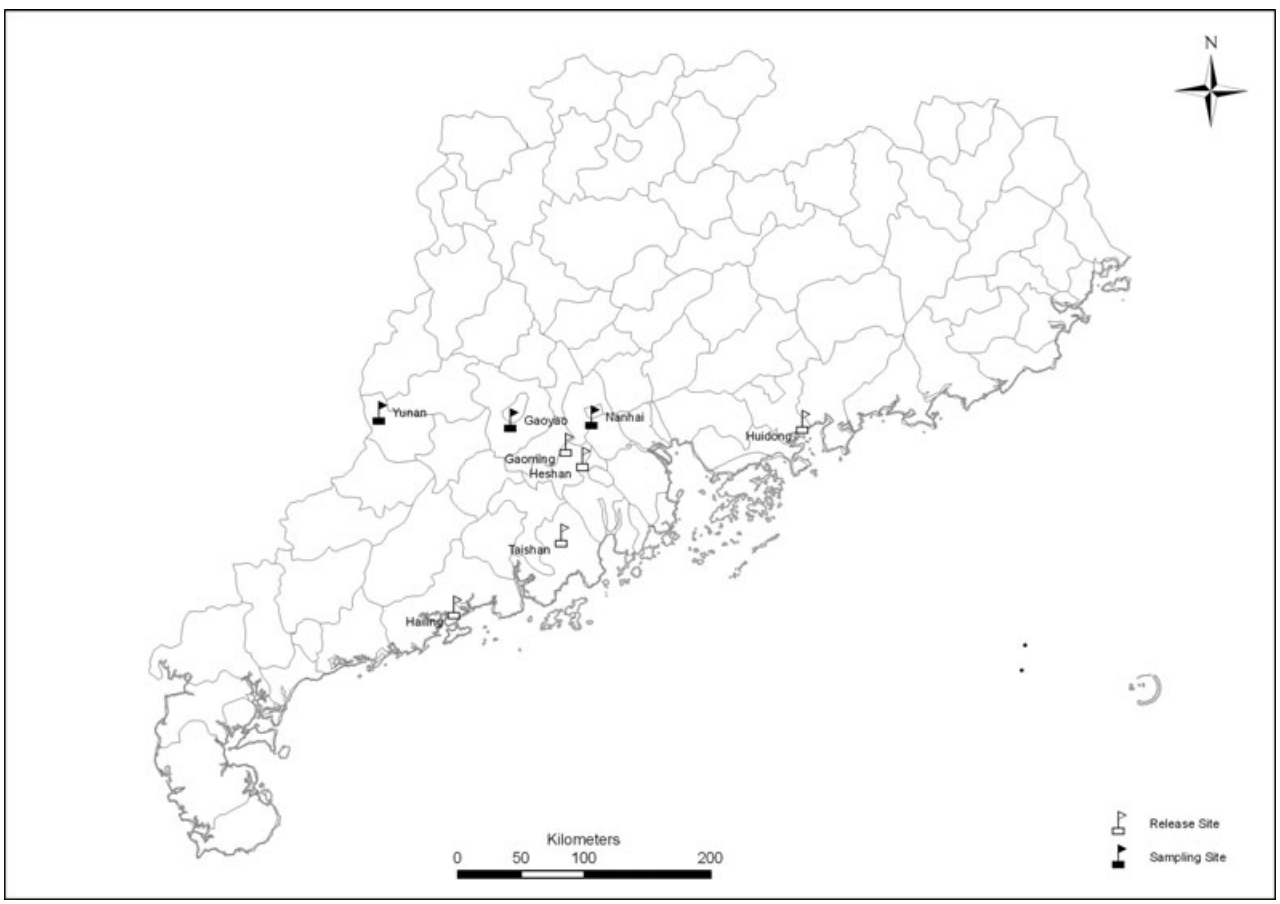

Fig. 2 Release and sampling sites in Guangdong Province for parasitoids of *Oracella acuta*. [GS (2009)1375]

Table 3 Releases of *O. acuta* parasitoids at slash pine plantation field sites in Guangdong Province, China.

Site	Year	Number of releases	Trees or caged branches	*Acerophagus coccois*		*Zarhopalus debarri*		*Allotropa oracellae*	
				Females	Males	Females	Males	Females	Males
Heshan	1996	2	5	12	16	16	12	29	20
	1997	4	34	0	0	80	23	113	96
Hailing	1995	1	7	0	0	26	7	0	0
	1996	1	1	0	0	7	3	0	0
	1997	4	11	0	0	56	22	27	23
Taishan	1997	21	105	0	0	525	413	322	264
	1998	7	36	275	236	0	0	0	0
	1999	8	42	250	165	286	128	0	0
	2000	3	15	29	10	0	0	0	0
Gaoming	1998	7	62	571	366	2	2	0	0
Huidong	2003	1	12	1 036	552	0	0	0	0
Total		59	330	2 173	1 345	998	610	491	403

The Gaoming and Heshan sites were 20–30-year-old pure slash pine plantations. *Acerophagus coccois* was the main parasitoid released at Gaoming, while all three species were released at Heshan. The Hailing site was composed of small slash pine plantations intermixed with Masson pine. *Zarhopalus debarri* was the primary parasitoid released at Hailing, although a few *A. oracellae* were released in a separate plantation at this site in 1997. The Taishan and Hailing plantations were on poor sites, and the pines were stressed. These four release sites had been infested for several years prior to parasitoid release. The Huidong site was added in 2003 in an effort to avoid interspecific competition with other natural enemies. This 23-year-old slash pine plantation was on the outer edge of the current range of *O. acuta*, and very few individuals of insect species associated with established *O. acuta* populations were evident. *Acerophagus coccois* was the only parasitoid species released at this site.

The *Z. debarri* release site at Taishan was moved in 2004 as the area was to be cleared for construction. In an effort to preserve any parasitoids on the trees, some 600 infested branches were cut and taken to a nearby slash pine plantation and tied to pine branches at the new site. At the original site, only half of the trees eventually were cut, with the other half preserved in an effort to maintain parasitoid populations.

Initial parasitoid releases in 1995–1996 were from glass vials attached to trees. Subsequent releases were into fine-mesh cages on trees or individual tree branches. Parasitoids were introduced into the cages, and the cages were removed 3 days later.

Parasitoid establishment monitoring

Establishment of parasitoids was checked by field collections of infested shoots. Shoots were randomly collected from pines in the release sites in the spring and the fall. The shoots were examined under a microscope and all mummified *O. acuta* placed in gelatin capsules. The shoots were then enclosed in cardboard boxes with a glass vial inserted into the top. Parasitoids collected in the gelatin capsules and vials were identified.

In 1998 and 2000, more systematic surveys were conducted at the Taishan release site. In May 1998, shoots were collected within the *A. oracellae* release plantation, and from pines at sample locations 10, 20, 250 and 500 m from the release site in each of the four cardinal directions. Five trees at each location were sampled by clipping eight infested shoots per tree. A similar survey was conducted in May 2000, but the sampling took place at 150, 250 and 500 m from the release plantation. In 2002, field collections of shoots were made in *O. acuta*-infested slash pine plantations near Gaoyao, Yunan, and Nanhai, areas not located near the parasitoid release sites (Fig. 2).

Results

Parasitoid shipments

A total of 19 972 parasitized *O. acuta* were sent to China in 49 shipments from 1996–2004 (Table 1). The percentage of mummies from which one or more

Table 4 Parasitoid emergence from *O. acuta* from infested slash pine shoots in the rearing facility in Guangzhou, China.

Year	Number of trees checked	Empty mummies	Intact mummies†	Gelatin capsules			Rearing boxes‡		
				Acerophagus coccois	*Zarhopalus debarri*	*Allotropa oracellae*	*Acerophagus coccois*	*Zarhopalus debarri*	*Allotropa oracellae*
1996	2	0	0	0	0	0	0	4	0
1997	53	12	27	0	3	14	0	9	4
1998	24	16	0	0	0	0	6	0	1
1999	53	1	0	0	0	0	0	0	0
2000	54	18	29	0	28	0	0	36	0
2001	3	20	90	0	0	39	0	0	1
2002	2	0	0	0	0	0	0	0	11
2003	7	0	0	0	0	0	7	0	0
2004	7	11	23	0	0	9	0	0	11
Total	205	78	169	0	31	62	13	49	28

†Mummies collected from shoots and placed individually in gelatin capsules.

‡Infested shoots placed in rearing boxes after all visibly mummified *O. acuta* removed.

individuals emerged ranged from 31.3% to 64.1%. Of the 15 430 emerging wasps, 12 933 were the three target species. Hyperparasitoids and unknown species accounted for the other 16.2% of the total emergence. *Acerophagus coccois* was the most prevalent primary parasitoid, representing 56.3% of the parasitoid emergence. The sex ratio of emerging parasitoids was skewed toward females for all three target species.

Parasitoid mass-rearing in Guangzhou

Just over 37% of the *O. acuta* primary parasitoids emerging from the shipped mummies were released in the rearing facilities (Table 2). Only 183 F1 parasitoids were reared from the 4 833 parasitoids released in the mass-rearing facility (Table 4). Ninety-three parasitoids emerged from the 169 intact mummies extracted from the shoots, although no *A. coccois* was collected from these mummies. The other 90 F1 parasitoids emerging were from shoots placed in rearing boxes following examination under a microscope. All *A. oracellae* F1s collected in 2001 were males.

Field releases and establishment monitoring

Almost half of the 6 020 parasitoids utilized at field sites were released in plantations near the Taishan seed orchard (Table 3). One hundred and eighteen wasps of the three imported species were collected during establishment checks, including 89 *Z. debarri* (Table 5). Most of the parasitoids were recovered from the Taishan area. In 1998, 23 *Z. debarri* and 6 *A. oracellae* were captured at Taishan, although neither species was released there that year. Three *A. coccois* were collected in 2002, 2 years after any releases of this species. One *A. oracellae* was collected at Taishan 5 years after that species had been released. Only two *A. oracellae* were collected in the field at Heshan, but these collections occurred 2 years subsequent to any releases at this site. Only one wasp of each imported species was recovered at each of the release sites at Hailing and Gaoming. No imported parasitoid species were collected at Huidong or at the three non-release sites.

Substantial numbers of parasitized *O. acuta* were collected at the Taishan release sites starting in 1998. Most of this parasitism was by the encyrtid *Anagyrus dactylopii* Howard, an Oriental species with numerous hosts (Noyes & Hayet, 1994). *Anagyrus dactylopii* had not been previously reported as a parasitoid of *O. acuta*. This species also was collected at other sites where mealybug infestations had been established for several years, and was the predominant species reared from mummies (Table 5). *Anagyrus dactylopii* is a gregarious parasitoid, and usually more than one parasitoid emerged from a mummified mealybug.

Despite the presence of predators and the prevalence of *A. dactylopii*, spring mealybug populations remained substantial. At the Taishan release sites, the number of fresh resin cells constructed by *O. acuta* females averaged 4.6 and 8.2 per new growth flush in 2002 and 2004, respectively. Numerous mealybugs also were evident on the new growth at the other release sites.

Table 5 Parasitoid emergence from shoots collected in slash pine plantations infested by *O. acuta* in Guangdong Province, China.

Site	Sampling years	Trees sampled	Shoots sampled	Empty mummies	Intact mummies	*Acerophagus coccois*	*Zarhopalus debarri*	*Allotropa oracellae*	*Anagyrus dactylopii*
Heshan†	1995–2004	396	2 805	260	270	0	0	2	83
Hailing†	1996–2004	944	5 454	1 856	1 019	0	0	1	0
Taishan†	1996–2006	1 692	8 763	1 481	1 639	13	89	12	2 073
Gaoming†	1998–2004	429	2 701	224	194	1	0	0	25
Huidong†	2003–2004	72	296	46	5	0	0	0	3
Gaoyao‡	2004	13	150	20	3	0	0	0	4
Yunan‡	2004	8	100	0	0	0	0	0	0
Nanhai‡	2004	4	50	2	1	0	0	0	2

†Parasitoid release sites.

‡Sites where no parasitoids released.

Discussion

Many attempts at classical biological control are not successful (Greathead & Greathead, 1992). Although *Z. debarri*, *A. oracellae*, and *A. coccois* provide effective control of *O. acuta* in the US, they have exerted no demonstrable impact on *O. acuta* populations in China in the 10 years since their first release. The capture of a few wasps several years after the last release at some sites indicates that these species are capable of reproducing and surviving in China. However, the low numbers captured suggest that these species are not firmly established to date. Continued monitoring is necessary to ascertain if populations of the three species continue to maintain a presence in the release sites and if their ranges expand. A systematic, standardized monitoring plan should be implemented to accurately document their population trends.

One reason for the unsuccessful biological control may be differences in *O. acuta* seasonal population levels between the US and China. In the US, *O. acuta* females are present on the shoots from late March through to November (Clarke *et al*., 1990). Population levels during this time of the year depend primarily on natural enemy populations. When parasitoid populations are not adversely affected by insecticide applications, they can quickly reduce mealybug numbers and maintain the populations at very low levels. In China, *O. acuta* populations increase during March through to June, then decrease dramatically in the summer. Numbers then rebound in September before most crawlers overwinter in December (Zhou *et al*., 2003).

The reasons for the summer population declines are unknown. Intraspecific competition could be a factor. High *O. acuta* populations can cover the entire shoot, leaving emerging crawlers with no room to settle. The crawlers may perish, or they could estivate through the summer until new growth flushes are available. Slash pines also may have fewer growth flushes than loblolly pines (Burns & Honkala, 1990), reducing the availability of feeding sites. There also are climatic differences in the infested regions in China and the US. The current range of *O. acuta* in Guangdong is between 21° and 25°N latitude, while the US range lies between 30° and 35°N latitude. Although mean summer temperatures in the two regions are similar, the mean annual rainfall in southern China is greater than in the southeastern US, at 173.8 cm and 126.3 cm, respectively (Li *et al*., 2005). In addition, the rainfall in the southeastern US is more evenly distributed throughout the year. The summer is the rainy season in subtropical China, with close to 40% of the annual rainfall occurring from June through to August. The heavy summer rains may negatively impact *O. acuta* populations in China. The mean annual rainfall in the Yangtze-Haihe River Basin, north of the current range of *O. acuta* in China, is similar to annual rainfall in the southeastern US, although the summer rainfall is still appreciably higher. The persistence of summer population declines should be examined once *O. acuta* expands into the loblolly pine plantations in this region. If mealybug hosts remain available throughout the summer, the opportunity for parasitoid establishment should increase.

The mid-summer population decline creates a lengthy period in which suitable hosts may be unavailable for the parasitoids. In addition, the seasonal population fluctuations may result in less overlap of mealybug generations throughout the year, which could leave emerging parasitoids without preferred life stages for parasitism. All three species prefer adult hosts, and *Z. debarri* utilizes adults almost exclusively (Sun *et al*., 2004a). Although *A. coccois* and *A. oracellae* may parasitize second and

third instars, the sex ratio of the emerging parasitoids may be skewed. Parasitoids may oviposit male eggs in small hosts and female eggs in large hosts (Jones, 1982). It is unknown if this behavior occurs in these two species, but in the 2001 *A. oracellae* release onto caged trees, only male offspring were produced.

Another possible factor in the failure of the parasitoids to exert control of *O. acuta* is interspecific competition. Tang *et al*. (1995) identified a number of potential predators and associates of *O. acuta* in China. Numerous species were noted on the infested shoots examined during this study and may have interfered with the parasitoids' abilities to locate and parasitize hosts. The imported parasitoids also face competition from *A. dactylopii*, which has alternate hosts that can be utilized during periods of low *O. acuta* numbers, allowing it to maintain moderate population levels. *Anagyrus dactylopii* could then respond quickly to the increased *O. acuta* numbers in the fall. *Acerophagus coccois* also has multiple hosts, but it is unknown whether this species has parasitized any other mealybugs in China, and if so, if these other hosts could improve its survival capabilities and affect its impacts on *O. acuta*.

The number of parasitoids released at each site may have been too low to facilitate survival and establishment. Some classical biological control efforts were successful after the release of limited numbers of natural enemies (Debach, 1974). However, most biological control programs for mealybugs utilizing imported parasitoids traditionally have released large numbers. Muniappan *et al*. (2006) released over 24 000 parasitoids within 1 year on Palau to successfully suppress populations of the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink. Infestations of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), were greatly reduced in the Imperial Valley, California after the release of 850 000+ parasitoids from 1999–2004 (Roltsch *et al*., 2006).

The ability to produce large numbers of parasitoids for release was incumbent on mass-rearing in China, but this program was not successful. The reasons for the inability of the mass-rearing facility in China to produce parasitoids are unknown. Initially the seedlings were of poor quality and supported few mealybugs. Improvements in the number and vigor of the pines used to maintain mealybug populations in the new rearing facility did not result in increased reproduction of the parasitoids. Adult *O. acuta*, the preferred life stage for progeny production for the parasitoids, may not always have been present within the rearing facility during parasitoid releases. Future considerations could be the establishment of field insectaries to rear parasitoids or utilizing *Anagyrus dactylopii* in the rearing facility to test methods of increasing parasitoid production.

The failure of the mass-rearing facility resulted in the use of parasitoids shipped from the US for field releases. Initially parasitoids were provided to China from the mass-rearing facility in Georgia which was maintained by a post-doctoral student. Upon his departure, parasitoid shipments from the US were dependent on field collections. These shipments generally were sporadic since no full-time collectors were available to continually locate mealybug infestations and collect mummies. It often was difficult to find sufficient numbers of mummies to ship, as detectable mealybug populations result from a lack of parasitism. There usually was a narrow window of opportunity to locate infested pine plantations in which parasitoid populations had recovered but had not yet caused the *O. acuta* population to collapse. Therefore, low numbers of parasitoids were available for shipment to China.

Biological control failures may result from insufficient resources (Van Driesche & Bellows Jr., 1996). Ideally, field studies to determine actual or potential effects of the climate in Guangdong Province on mealybug and parasitoid populations would have been completed before or during the releases. However, the Pest Control Station responsible for the parasitoid mass-rearing, field releases, and monitoring in China had numerous other pest management issues to address. For example, the pinewood nematode and the associated pine wilt disease were causing widespread mortality of pines. Therefore, funding and research for the biological control of *O. acuta* was not always a high priority. A further complication was a 3-year cooperative agreement between the US and China that coupled the *O. acuta* program with a biological control program for hemlock woolly adelgid, *Adelges tsuga* Annand, in the US. This agreement resulted in a rush of parasitoid shipments to China before the mass-rearing facility was fully ready and the necessary data on climatic compatibility were gathered. In the US, the only scientist fully dedicated to the project was a post-doctoral student from 1995–1997. A fully funded and staffed program is likely necessary to address questions associated with the mass-rearing and field establishment of parasitoids for *O. acuta* in China. These problems illustrate the importance of the adoption of the new guidelines for classical biological control programs established by the International Plant Protection Convention (IPPC, 2005).

Given the difficulty of collecting parasitoids in the US and the inability to rear large numbers of parasitoids in China, the classical biological program for *O. acuta* was suspended after 2004. Resumption of the program is dependent on increased research and allocation of resources. If the mealybug infestation expands into loblolly pine

plantations, the potential economic impacts may spur renewed efforts to investigate *O. acuta* biology in China and improve methods for mass-rearing. There is also the potential for one or more of the imported parasitoids to adapt to the conditions in China and begin to impact *O. acuta* populations. *Anagyrus dactylopii* may develop a stronger search image for *O. acuta* and increase parasitism rates. Augmentive biological control using *A. dactylopii* could be attempted if mass-rearing techniques are perfected.

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