Fate of *Cyanotricha necyria* (Lepidoptera: Notodontidae) and *Pyrausta perelegans* (Lepidoptera: Pyralidae) Released for Biological Control of Banana Poka (*Passiflora mollissima*) on the Island of Hawai'i

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ABSTRACT. The establishment and fate of 2 newly released biological control agents, *Cyanotricha necyria* (Lepidoptera: Notodontidae: Dioptinae) and *Pyrausta perelegans* (Lepidoptera: Pyralidae), on their host plant, banana poka, *Passiflora mollissima*, were monitored for 9 months in the Ola'a rainforest on the Island of Hawai'i. Three months after release, *P. perelegans*, a bud feeder, had initially become established. It remained present throughout the study period despite an initial egg parasitism level of nearly 60%. Although 4.5-fold more *C. necyria* individuals were released than those of the bud feeder, *P. perelegans*, it did not become established. This was due in part to the effects of arthropod predators and parasitoids, but more to the high level of pupal removal (probably by birds) and possible lack of a nectar resource for the adults.

INTRODUCTION

Banana poka (*Passiflora mollissima* (HBK) Bailey), a perennial vine from the Andes mountains in South America, has invaded many hydric and mesic upper elevation forests on the islands of Hawai'i and Kaua'i (LaRosa 1992). This vine is a state-listed noxious weed and is generally considered the most serious weed in upper-elevation Hawaiian forests. It forms dense canopies capable of smothering trees and radically altering wildlife habitat. In February 1988, following host-testing and final approval for release, field efforts on biological control of banana poka began with the first release of *Cyanotricha necyria* (Felder & Rogenhofer) (Lepidoptera: Notodontidae (Dioptinae)) in the Laupahoehoe Forest Reserve on the island of Hawai'i (Markin et al. 1989). Releases of eggs and larvae eventually totaled >10,000 individuals and these completed development to adult (Markin, unpubl. data). However, 2 years of post-release surveys failed to show any signs of subsequent generations (Markin, unpublished data). As a result, it became desirable to closely monitor released individuals to determine if establishment would occur or the reasons for non-establishment.

Following the 1988 releases of *C. necyria*, *Pyrausta perelegans* Hampson (Lepidoptera: Pyralidae) was host-tested at the Hawaii Volcanoes National Park Quarantine Facility (HVNPQF) near Volcano, Hawai'i (Markin, unpubl. data). It was considered by Rojas de Hernandez and Chacon de Ulloa (1982) to be of major economic importance in cultivated banana poka ("curuba") plantings in Colombia. It was also "the most interesting insect found" in a 1982 Andean survey of *Passiflora* insects by Pemberton (1989), who noted that it caused large numbers of damaged and aborted buds in some *P. mollissima* populations. Whereas *C. necyria* was a defoliator, this insect was primarily a bud feeder, important because fewer buds would mean fewer fruits and thus fewer seeds available for spread by birds and feral pigs. *Pyrausta perelegans* was approved in early 1991 for release on banana poka and was monitored along with *C. necyria* in this study.

This paper reports the outcome of releases and fate of both insects as determined during 9 months of continuous monitoring in 1991. Our main interest was on identifying factors responsible for limited or non-establishment of one or both insects. Historically, many

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biological control releases have not been intensively monitored. Given the time and money involved in testing candidate biological control agents, it would be informative to study released populations, especially if reasons for their non-establishment can be determined. This information is pertinent to all biological control of weeds programs because success has been estimated at only 55% worldwide (DeBach 1974) and 50% for Hawai'i (Markin et al. 1992).

MATERIALS AND METHODS

Release/Study Area

The 0.65 ha release/study area was located in a tropical montane rainforest at ca. 1232 m on the island of Hawai'i. It consisted of idle land at the University of Hawaii Volcano Experimental Farm (UHVEF), adjacent private land, and adjacent land in the Ola'a Tract of Hawaii Volcanoes National Park (HVNP). This area was chosen for its limited, yet convenient access and its abundant, yet accessible banana poka vines. Enough vines hung from tree ferns and small trees to allow tagging and close examination from the ground. Three hundred twenty-one vines were initially tagged; later in the study it was possible after the installation of a ladder to tag an additional 20 vines located 4–7 m above the ground in a thick banana poka canopy. All tagged vines had an actively growing tip and at least 10 expanded distal leaves. If a tagged vine was determined to be substantially defoliated after the development of one or both biological control agents was completed, or if disease had caused leaf drop, the numbered tag was moved to an adjacent vine.

Cyanotricha necyria Acquisition and Release

The immediate progenitors of the *C. necyria* individuals ultimately released in this study originated as pupae in the Andes mountains at 2743 m in the vicinity of Ipialis, a town in western Colombia on the Ecuador border. Three shipments of pupae were made to HVNPQF beginning in January 1991. When adults emerged in sleeve cages (43.1 x 43.1 x 53.3 cm), a honey based solution and banana poka cuttings were provided for nutrition and oviposition, respectively. Eggs were transferred to an adjacent building and, upon eclosion, larvae were reared until they were released as late second instars. Before release, samples of these larvae and field-collected pupae were submitted to insect pathologist G.M. Thomas, Consulting Diagnostic Service, Berkeley, California, and both samples were found to be free of entomopathogens.

The first release of *C. necyria* in the release/study area, though not the first release in the state, occurred on 27 February 1991. A cohort of seven larvae, mostly second instars, was placed with a fine-tipped brush onto the fourth or fifth expanded leaf of tagged vines. An initial 3,031 larvae, mostly second instars, were released between 27 February and 24 April 1991; 2040 were added between then and early November for a total of 5,071. From October to early November, 542 large larvae (mostly fourth instar) were among those released in order to have more larvae survive to the end of the ultimate fourth instar and eventually produce cocoons on tagged vines. In early summer, 156 adult moths from HVNPQF were released into a thick, draping, banana poka canopy located on UHVEF land. The sum total released of all stages was 5,227 individuals (5,071 larvae + 156 adults); more than 4.5-fold the sum total of *P. perelegans* released.

Pyrausta perelegans Acquisition and Release

The immediate progenitors of the *P. perelegans* individuals ultimately released in this study originated as eggs and small larvae collected in the Andes mountains at 2743–3048 m in the vicinity of Merida, a town in western Venezuela. The first collection was made in December 1990 and was followed by 2 more collections in 1991. This stock was shipped to HVNPQF. Conditions at this facility are maintained at near-ambient tem-

perature and humidity (Markin et al 1989). Neonates and field-collected larvae were reared individually on folded banana poka leaves in large plastic petri dishes (2.5 x 12.7 cm diam.) and the resulting pupae were placed in open paper cartons (8.8 x 8.8 cm diam.) within sleeve cages (43.1 x 43.1 x 53.3 cm). Emerged adults were provided with a honey based solution for nutrition and banana poka cuttings for oviposition. Leaves with eggs attached were removed, dipped in a 1% chlorine bleach solution, rinsed in running water, blotted dry, and placed in large plastic petri dishes (2.5 x 12.7 cm diam.). For rearing, petri dishes were transferred from HVNPQF to an adjacent laboratory building where the disease potential was considered low because larvae had not been reared there previously. Conditions in this building also approximated ambient. When eggs hatched, larvae were reared as stated above until they were released as late second instars. Before release, a sample of these larvae was submitted to G.M. Thomas and found to be free of entomopathogens.

The first release of *P. perelegans* in the State of Hawaii occurred 22 February 1991 in the release/study area. Late second instars were placed individually onto large, bract-enclosed, banana poka buds with a fine tipped-brush. Because vines had been tagged prior to larval release and the number of larvae exceeded the number of tagged vines, both tagged and non-tagged vines were used in order to provide sufficient buds for larval placement. Only 1 larva was placed per tagged vine, but non-tagged vines having many large buds received 2 or 3 larvae. Five hundred thirty larvae were released between 22 February and 4 March 1991; 192 more were added between then and late May for a total of 722. In July 1991, banana poka leaves from HVNPQF with a total of 404 eggs/neonates were wired onto vines in a thick, draping, banana poka canopy at a single location on UHVEF land. The sum total released of all stages was 1,126 individuals.

Monitoring of Both Species

Weekly monitoring of tagged vines began in early March 1991 as they received larvae to document whether or not initial, and more importantly, permanent establishment would occur. The initial post-release check of *C. necyria* took place 8 days after first release and involved counting larvae (live and dead) on the first ten distal leaves, and then further up the vine until a branch point was reached, or until the leaves could no longer be searched by hand or visually. The first check for *P. perelegans* occurred 12 days post-release and consisted of damaged-bud counts. Before the end of March, weekly monitoring involved counts of both species' larvae on leaves within the distal/proximal vine portions; the number of bract-enclosed buds occupied by *P. perelegans*; and the total number of bract-enclosed buds present.

Little disturbance was imposed on individuals of any life stage of either species, whether on leaves or within buds. Most ventral leaf surfaces could be viewed for eggs and larvae by manipulating internodes. Occupied buds could be confidently ascertained by looking for a single silked-over entrance hole in the bracts. Rarely did a bud require opening to confirm the presence of *P. perelegans*. *Pyrausta perelegans* larvae were mostly left undisturbed during the study, as only 3 individuals were removed to the laboratory. Adults of both species were sought periodically by walking under banana poka canopies and viewing the ventral surfaces of the leaves. *Cyanotricha necyria* adults, being diurnal, were also sought in flight above vine canopies.

In addition to determining the establishment status of the biological control agents, monitoring included detection of potential biotic factors responsible for limiting or preventing establishment. When examining tagged vines, all dead or dying *C. necyria* larvae found were collected and subsequently frozen. Subsamples totaling 28 *C. necyria* larvae were submitted to G.M. Thomas for detection of any entomopathogens.

Predation by arthropods on *C. necyria* larvae was recorded when observed and predators captured if possible. *Pyrausta perelegans* larvae were not observed for direct predation because they are normally within buds. Any arthropods that externally fed as saprophages on *C. necyria* or *P. perelegans* larvae were noted and captured when possible.

The number and location of *C. necyria* cocoons on tagged vines were recorded to monitor non-arthropod predation of pupae. Cocoons found on non-tagged vines and other plant species were marked with inconspicuous wire loops placed near to them and the fate of these pupae was likewise monitored.

To study egg parasitism, the location and condition of both species' eggs were recorded weekly to monitor their fate. The few (7) *C. necyria* eggs located were allowed to remain in place. Entire leaves or leaf portions with darkened (parasitized) *P. perelegans* eggs were collected and taken to the laboratory where the eggs were removed on leaf discs cut by a cork boring tool and held in gelatin capsules for parasitoid emergence.

From April through December 1991, 261 *C. necyria* larvae (mostly fourth instars) and 22 *C. necyria* pupae were recovered from released stock and returned for laboratory rearing of parasitoids. Individuals had been in the field for at least 2 weeks before recovery and recovered individuals did not include those larvae which had been released as fourth instars. Parasitic wasp pupae found in association with *C. necyria* larvae (or where larvae had been observed during the previous visit) in the field were recorded by location (tagged vine #), collected, and held for emergence in petri dishes (1.2 x 9.5 cm diam.) in the laboratory.

RESULTS

Probable Non-Establishment of *Cyanotricha necyria*

The first post-release check (7 March) of *C. necyria* showed that, on the 35 tagged vines each of which had received a cohort of seven larvae 3–8 days earlier, all had 1 or more larvae present on the vine. Most of the larvae were still distally located (first through tenth leaf) on the vines and this pattern continued during the study (Table 1). Substantial defoliation occurred when most of a cohort survived to or through the ultimate fourth instar.

Table 1. Monthly mean number of bract-enclosed banana poka buds per tagged vine in Ola'a forest study area, Volcano Hawai'i, 1991.

	Months 1991									
Value	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean	2.0	1.6	1.9	2.0	2.1	1.5	2.4	2.7	1.9	0.9
Std Dev	2.4	2.3	2.7	2.4	2.4	2.2	2.5	2.5	2.1	1.6
Range	0–11	0–12	0–11	0–11	0–12	0–17	0–15	0–12	0–14	0–9
Vine observations	418	1048	1283	963	1002	1023	1465	1594	1363	340

By the end of the study in December 1991, after at least 10,499 observations of vines, there was no evidence of establishment in the form of a subsequent, successfully developing, generation. As the following data indicate, recovery of stocked individuals occurred, sometimes in a later life stage, but this was not considered establishment. At least 93 of the stocked larvae successfully pupated on tagged vines, non-tagged vines, and other plant species. Taking into account the 22 pupae removed to the laboratory, plus 3 attacked and 57 missing pupae, only 11 of the 93 pupae eventually produced adults in the

Table 2. Observed location of stocked *C. necyria* larvae on tagged banana poka vines in Ola'a forest study area, Volcano Hawai'i, 1991.

Vine Section	Percentage of vine observations with vine section having (X) *C. necyria* larvae[1]							
	0	1	2	3	4	5	6	≥7
Distal[2]	7.0	36.7	17.3	11.6	8.2	8.1	5.9	5.1
Proximal[3]	79.5	15.0	4.1	1.3	0.1	0.0	0.0	0.0

1. n = 1557 vine observations.
2. First 10 expanded leaves back from the tip.
3. Eleventh leaf and beyond, until branching point or visual limit.

field. Only 1 of the adults was ever seen; it was on a tagged vine. A total of 7 eggs was detected during the 9-month study. They were found on 2 occasions (July, October), but failed to hatch and likely were infertile, as they eventually collapsed. From the 2 releases of a total of 156 adults, the singular result was the observation of several individual flights at the top of the banana poka canopy at the release point 1 or 2 days after each release. These horizontal flights were glimpsed only briefly, but there was no apparent contact with the vines by the moths.

The host plant resource for *C. necyria* [i.e., the distal leaves 15–40 cm back from the tip used for oviposition as well as larval feeding (Markin et al. 1989)] was enormously abundant due to the number of vines present in the release/study area. Because of this, it is clear that a lack of host plant resource did not exist and, therefore, could not have contributed to the probable non-establishment of *C. necyria*.

No evidence was gathered to suggest competition by *P. perelegans* as a reason for probable non-establishment of *C. necyria*. Only 1 *C. necyria* larva-occupied leaf was found upon which *P. perelegans* had oviposited, resulting in the apparent consumption of some of the latter's eggs. Otherwise, no other vine was found occupied by both species. The virtual lack of the 2 species being found together concurrently, plus the likelihood that bud feeding by *P. perelegans* did not affect leaf availability or host suitability for *C. necyria*, strongly suggests that competition between the 2 species did not take place and lead to the probable non-establishment of *C. necyria*.

Establishment of *Pyrausta perelegans*

The first post-release check (6 March) of *P. perelegans* showed that 55% of the 139 tagged vines which first received a single larva had 1 or more bract-enclosed buds destroyed. Because this species' larva actively searches for a suitable bud, it is likely that many moved off of their original vines without causing any damage. The possibility that some larvae were preyed upon before finding a bud cannot be discounted, but this was not investigated. At the end of April 1991, 2 adult females and 4 eggs of *P. perelegans* were detected. The nocturnal adults were found at rest on the ventral surfaces of banana poka leaves. Afterwards, only eggs and larvae were detected during the study. Eggs were generally found several to a leaf on expanded distal leaves (the first through tenth), though occasionally more proximal, and rarely on bract-enclosed buds.

The initial recovery of a non-stocked larva was found at the end of May, when a tag was moved to a new vine and an occupied bud was found. About a week later, 6 more larva-occupied buds were found several meters away on vines that had not been included in earlier larval releases. This additional find confirmed initial establishment of a subse-

quent, successfully developing, generation. Larvae continued to be detected periodically through the remainder of the study, but always at a low level. For example, 1 or 2 larvae were detected on just over 100 tagged vines on at least 15 occasions when released larvae would no longer have been present. The greatest level of occupancy detected on any occasion was 5.7% (6 vines out of 105 vines).

Host-plant resources for *P. perelegans* (i.e., distal leaves for oviposition and bract-enclosed buds for early larval development) were in abundance at all times. Distal leaves were always present due to the vines' normal continual growth. Occasionally, some vines experienced partial leaf loss from an anthracnose, but defoliation was never widespread throughout the release/study area. A monthly average of about 2 bract-enclosed buds per tagged vine were infested in the study (Table 2). The range of bud numbers for vines was as high as 17 in August, but was generally in the 0–12 buds/vine range.

Factors affecting establishment

Disease was not detected as a mortality factor throughout the study. Subsamples of field-recovered, dead, *C. necyria* larvae (n = 28) and unemerged pupae (n = 6) yielded no entomopathogens through analysis. An unidentified microsporidian had been a serious problem in stock received previously from Colombia. This entomopathogen caused >95% mortality by the time fifth generation larvae were produced in HVNPQF (Markin et al. 1989).

Predation by arthropods was directly observed within days of the first *C. necyria* release. Four dead larvae were found on 1 vine, 2 of which were impaled on the beaks of nabid nymphs, which were commonly observed on banana poka leaves. Two instances of predation by thomisid spiders were also observed. Markin et al. (1989) had previously reported that lycosid spiders attacked small larvae. A chrysopid larva was seen feeding on a small larva. A total of 67 *C. necyria* larvae, or about 1% of the total released, were found dead (both intact and damaged) on banana poka leaves. Adults of *Forcipomyia* (*Euprojoannisia*) *hardyi* Wirth & Howarth (Diptera: Ceratopogonidae) were observed feeding singly on live larvae on 4 occasions. The larvae exhibited no debilitating injuries from the loss of haemolymph and continued normal development after being removed to the laboratory. Dead larvae, not always intact, were also observed being fed upon by 1 or 2 ceratopogonids on 5 occasions.

Predation by non-arthropods, almost certainly birds, seriously affected *C. necyria* pupal survival. Pupae that appeared to have a peck hole were found inside cocoons bearing a similar hole. Other pupae disappeared completely from damaged cocoons. The only insects perhaps capable of removing pupae from their cocoons, introduced vespids, were rare in the rainforest environment. Of the 93 pupae monitored *in situ*, including 22 (24% of total) eventually taken to the laboratory, 3 (3%) were attacked, 11 (12%) emerged, and 57 (61%) were removed from their cocoons by predators. When the 22 pupae removed to the laboratory were excluded, the 3 pupae attacked represented about 4% of the monitored pupae, those that emerged about 15%, and those removed by predators about 80%.

Parasitism was not detected in the 7 *C. necyria* eggs found. Egg parasitism also was not detected in about 1,700 field-collected eggs sent from Colombia to HVNPQF (Markin et al. 1989), in eggs collected and observed in Colombia (Casanas-Arango et al. 1990), nor in HVNPQF-produced eggs placed in the field on Hawai'i and returned to the laboratory for examination (Markin, unpubl. data).

The parasitism level of released *C. necyria* larvae, based on 38 parasitoids obtained though rearing 261 field-recovered larvae and eight parasitoid cocoons found on larvae-stocked leaves, totaled 17%. A braconid species, *Meteorus* possibly "sp. near *ictericus*", was the sole parasitoid discovered.

Parasitism of *Cyanotricha necyria* pupae, based on 2 ichneumonid specimens, *Pterocormus cupitus* (Cresson), reared from 22 field-recovered pupae, was 9%. Ten to 100 percent of field-collected pupae and late-instar larvae sent from Colombia to HVNPQF were parasitized by the ichneumonids, *Coccygomimus pepsoides* Porter and *Ichneumon* sp., and a torymid, *Perissocentrus* sp. (Markin et al. 1989). Casanas-Arango et al. (1990) reported that 2 species of ichneumonid (not identified) emerged from *C. necyria* pupae in Colombia.

Though *P. perelegans* larvae were not observed for direct predation, because they are normally within buds, an unusual find documented that they can be subject to predation when leaves, rather than buds, are used for food. One dead *P. perelegans* larva, a released individual, was apparently killed by a syrphid larva found with it in such an atypical situation: a banana poka leaf that the caterpillar had tied together with silk.

Parasitism of *P. perelegans* eggs occurred among the first eggs laid and continued throughout the study. An endemic trichogrammatid wasp, *Trichogramma perkinsi* Girault (or near), was the first and most common of the 2 parasitoids reared; the other was *T. chilonis* Ishii, one of the most common trichogrammatid species in Hawai'i. Of a total of 167 monitored eggs, 56% were parasitized, 26% successfully eclosed, 4% collapsed or became damaged, and 14% were inconclusive as to their fate.

As previously indicated, very few *P. perelegans* larvae were disturbed by *in situ* examination. However, 1 developing flower was found occupied by a dead larva and an adjacent parasitoid cocoon inside the floral tube, but the adult wasp failed to emerge. Larvae were reportedly parasitized by an unidentified braconid wasp in Colombia (Rojas de Hernandez & Chacon de Ulloa 1982).

DISCUSSION

The non-establishment of *C. necyria* can be partially explained by the combination of predation and parasitism caused by arthropods. Our study has documented that removal of 60–80% of monitored pupae by non-arthropods occurred, which would likely hinder if not prevent establishment. If similar predation pressure exists for larvae, which are highly exposed and non-cryptic, then even fewer individuals would survive to establish the species. The Japanese white eye, *Zosterops japonica japonica* Meyr, one of the most common birds in Hawai'i and the only species frequently seen foraging on banana poka vines, was suspected of removing pupae and larvae.

The few adults of *C. necyria* that emerged may have encountered a lack of food. In its native range, the moth has been observed feeding on composite flowers in the genus *Baccharis* and on the bloom of an unidentified plant in the Papilionaceae (Casanas-Arango et al. 1990). In the release/study area, which was reasonably typical of Hawaiian environments infested with banana poka, nectar sources other than the native 'ohi'a tree (*Metrosideros polymorpha* Gaud.) and the introduced Asian raspberry (*Rubus ellipticus* Sm.) were not common or obvious, and may not be routinely available or suitable as nectar sources. Thus, the major nectar source group in the native habitat (i.e., Asteraceae) were scarce in the release area, which could ultimately prevent establishment regardless of the other factors. Obviously, a nectar source exists for the nocturnal *P. perelegans*, but its identity is unknown and it may not be available and/or suitable for the diurnal *C. necyria*.

Pyrausta perelegans became initially established in the release/study area approximately 3 months after the original release and it was found through the remaining 6 months of this study. Because *P. perelegans* requires about 3 months to develop from larva to adult, depending on temperature (Rojas de Hernandez & Chacon de Ulloa 1982), individuals found near the end of 1991 would have represented at least the third generation

produced in the release/study area. Within 3 months after this study's end, informal observations indicated that "a lot of bud damage" existed in the release area and larvae were being found with little or no effort (V. Tanimoto, pers. comm.). It continued to be found thereafter and was estimated to be causing destruction of about 5% of the banana poka buds in the general release area more than 2 years after introduction (Markin, unpubl. data).

Establishment of *Pyrausta perelegans* occurred despite substantial egg parasitization by *Trichogramma* spp. This represents a rarely documented case where an endemic insect (*T. perkinsi*) has presumably benefitted from the intentional introduction of an unassociated biological control agent into its environment. Howarth (1991) cites examples where biological control agents have reduced or extirpated non-target native insect populations, but here an unassociated biological control agent became a host for a native insect.

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