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Failure in Hawaii of the Sawfly, *Priophorus morio* (Hymenoptera: Tenthredinidae), A Biological Control Agent for *Rubus argutus*, Due to a Virus

George P. Markin and Roddy F. Nagata

Pacific Southwest Forest and Range Experiment Station, U.S. Department of Agriculture Forest Service, 1643 Kilauea Avenue, Hilo, HI 96720, USA

The sawfly *Priophorus morio* was introduced into Hawaii in 1966 to control the introduced blackberry *Rubus argutus*. The insect readily established and dispersed through the range of blackberry, but has since remained at very low population levels. A resurvey in mid-1980s of insects introduced against *R. argutus* indicates that the sawfly was not attacked by any parasites or known predators, but was heavily infected by a cytoplasmic polyhedrosis virus. Release records showed–and a survey confirmed–that *P. morio* had never been introduced to the island of Kauai. A disease-free strain was developed and released there in 1988. The new population underwent an explosive increase in numbers, rapidly dispersed through the range of *R. argutus* and appeared destined to become a successful biological control agent for this weed. Unfortunately, in <2 yrs, the virus appeared on Kauai, and reduced the population to very low levels. The virus is specific to the sawfly and has been recovered in the original sawfly population in Oregon in northwestern United States. It is believed that lax screening allowed the virus to be introduced with the original sawfly population–an omission that has destroyed the effectiveness of an otherwise very promising agent.

Introduction

The blackberry, *Rubus argutus* Link (Rosaceae) (previously referred to as *R. penetrans* Bailey) (Wagner *et al.* 1990), was introduced into Hawaii around 1894, probably for its desirable fruit. It is now established on at least 4 of our islands at elevations between 1,000 and 1,500 m (Neal 1965), the elevation that also contains most of the remaining native indigenous forests. The plant is readily spread by birds feeding on its fruit and once established, even in relatively undisturbed forests, it forms thick, impenetrable stands that interfere with access and with forest management. The plant was originally a problem in pasture lands, but in the 1960s the Hawaii Department of Agriculture (HDOA) introduced and released from North America 5 potential biological control agents (Julien 1987) of which 3 defoliators became established: *Croesia zimmermani* Clarke (Lepidoptera: Tortricidae) (Davis and Krauss 1965); *Schreckensteinia festaliella* Hubner (Lepidoptera: Heliodinidae) (Davis and Krauss 1964); and *Priophorus morio* (Lepeletier) (Hymenoptera: Tenthredinidae) (Davis and Krauss 1967).

The introduction of *P. morio* into Hawaii is believed to be only the second time that a sawfly has been used as a biological control agent; the first was an unsuccessful attempt to establish *Ucona acaena* Smith (Hymenoptera: Tenthredinidae) in New Zealand in 1936 (Miller 1970, Julien 1987). *P. morio* is a parthenogenetic sawfly with a holarctic distribution that extends through North America and Eurasia, where it is reported to attack a wide range of *Rubus* species. Besides having been deliberately introduced into Hawaii, it has been reported to have accidentally reached New Zealand and Australia (Callan 1978). In Hawaii *P. morio* was considered a good candidate for biological control since there were no known parasites that might affect it. Currently in Hawaii, a large complex of intentionally, or accidentally, introduced Hymenopterous parasites attack introduced Lepidoptera and are believed to interfere with their effectiveness as biological control agents (Howarth 1983). A sawfly could fill the same defoliator niche, but with no native representatives of its family in Hawaii and only a single other accidentally-introduced sawfly, the bristly rose slug *Cladius difformis* Panzer (Hymenoptera: Tenthredinidae), it should not have encountered a complex of specialized parasites.

By 1984 the general conclusion was that in open pasture lands, *R. argutus* was no longer a problem probably due to the combined impact of the three agents and pressure from grazing. In the forest environment, however, dense stands could be found, and they appeared to be spreading, particularly in disturbed areas. In 1984-5, a survey was conducted to determine the relative abundance and distribution of the three insects in the forest environment (Nagata and Markin 1986). The results of this survey indicated that *C. zimmermani* was the dominant agent followed by *S. festaliella*. *P. morio* was found only in very low numbers–a somewhat unexpected situation since no parasites were found attacking its eggs or larvae. These low numbers were tentatively concluded to be the result of a predator or disease. However, predator attack seemed less likely because of a lack of general predators such as ants and also because the other two Lepidoptera defoliators feeding on the same foliage should be equally susceptible, but were much more abundant. A disease was suspected of being the cause when 62% of the sawfly larvae brought into the laboratory for parasite rearing died within 10 d, usually showing symptoms of a virus-caused disease: halting of feeding; liquid being discharged from the anus (which would streak the Petri dishes as larvae wandered around); but no melting of the body after death. The presence of a virus was confirmed by a consultant pathologist, Gerald Thomas of Diagnostic Laboratory at Berkeley, California, who identified the agent as a cytoplasmic polyhedrosis virus.

Subsequent surveys of natural populations of the sawfly at 3 localities in the Willamette Valley of Oregon in 1991, the origin of most of the original populations introduced into Hawaii, has shown that over 50% of the larvae collected died of a similar virus disease with the same symptoms and incubation period. Detailed diagnostic tests to confirm that the 2 viruses are the same have not been conducted, but it is presumed that they are the same and that it was accidentally introduced into Hawaii along with the original colony of sawfly. The low population levels of *P. morio* and the high frequency of the virus have led us to conclude that this is probably the factor suppressing the effectiveness of this agent.

An opportunity to test this theory presented itself during the general survey for *Rubus* insects in 1984-5. When no sawflies were recovered and no typical sign of their feeding was found on the island of Kauai, we reviewed the HDOA records which confirmed that this insect had never been released on that island. If this virus is a limiting factor and a disease-free strain of the sawfly could be developed, introduced and established on Kauai, the population should increase to a much higher level than that found on the other islands, possibly reaching the level where it would be an effective control agent. In 1988, this experiment was undertaken in cooperation with the HDOA.

Biology

P. morio is a typical tenthredinid sawfly whose late instar larvae are approximately 15 mm in length with a striking, large, dark head, and white body with 2 dorsal gray stripes. Females are parthenogenetic and within 48 h of emerging, lay up to 20 single eggs in the tissue of the petioles of older *Rubus* leaves. Larvae are defoliators and can usually be found on the under sides of older leaves. The larvae in Hawaii pass through six instars and require 40 d to complete development in summer. Pupation occurs in a silk cocoon spun in the leaf litter under the plants. In Oregon, the sawfly overwinters as a prepupa, but in the milder climate of Hawaii, larval activity continues all year round.

The Virus

The pathology of the virus has not been studied in detail, but from our observations it appears to spread from larva to larva by consumption of contaminated foliage. In the laboratory, artificially infected larvae ceased feeding after 3-4 d and were dead by 5-10 d. Their frass is contaminated by 3 d, as is the liquid anal discharge, which begins at 4 d. In the field the virus is probably spread to new foliage by these two sources as well as by deterioration of the bodies of the dead larvae. Insect cytoplasmic viruses are poorly known, but appear to be very highly specific, attacking only a single species. However, we considered the possibility that the virus may not have been introduced with *P. morio*, but may have been an accidental crossover from *C. difformis*. However, of the 20 *C. difformis* collected in the field, none died of a virus. Of 25 laboratory-reared *C. difformis* fed foliage treated with *P. morio* virus, none died, as opposed to 100% mortality of a similar-sized sample of *P. morio*.

Insect cytoplasmic viruses are not known to be latent (or to have alternate carriers) which we presume was the situation with our virus, and was the basis we used to develop a virus-free strain. Field-collected larvae were isolated in individual Petri dishes and fed virus-free foliage obtained by propagating root cuttings of *R. argutus* in sterile media in a greenhouse at sea level in Hilo, Hawaii, the same location where the colony of virus-free sawflies was propagated. The location was isolated from the nearest other naturally-occurring *R. argutus* plant by 900 m in elevation and 30 km. Dead sawflies were discarded and those that survived were used to propagate the subsequent generation and repeat the process. During propagation no sign of the virus was found in the second and third generations reared in the laboratory, but to test for possible latency of the virus, subsamples were removed from the fourth generation and stressed by either rearing them under cold or very warm conditions, or starving them. None of these stressed larvae developed signs of the virus and the fourth generation was concluded to be virus-free.

The Release Area

The major significant *R. argutus* infestation on the island of Kauai is a 2000 ha plateau on the west end of the island between 960 m and 1200 m elevation. The area includes Kokee State Park, and is heavily forested, predominantly with introduced trees.

The area of release was on the east side of the infestation in a triangular 20 ha block of land enclosed by three roads at an elevation of 1140 m. The flat area enclosed is locally identified as the "berry flat trail head" and is located 1.7 km due east of Kokee Park headquarters. The area was chosen because it had some of the densest stands of *R. argutus* encountered in the state, probably as a result of the opening of the forest canopy by a hurricane in 1982 and subsequent disturbance by salvage logging. Releases were made at five separate locations within this 20 ha plot, with approximately 200 larvae or pupae being released at each of the 5 sites.

Post-release sampling was made by visiting the five release sites and lifting up individual shoots and looking on the undersides of the leaves for larvae. The observers soon learned to spot new feeding damage of the larvae and knew which shoots to look at. This visual search was conducted for 15-min intervals by two people and the figure converted to the number of *P. morio* larvae found in 30 mins of search for each release site. All larvae encountered were isolated in the field in individual Petri dishes along with the foliage on which they were feeding. These insects were returned to the laboratory, transferred to clean foliage, and observed for symptoms of the virus.

Post-release monitoring was conducted only at the 5 release sites for the first year, but after establishment was confirmed, 10 additional sampling sites were selected at various locations throughout the 2,000 ha *R. argutus* infestation. All sites were sampled at least twice a year, approximately in spring and late fall. As a control for these samplings, 3 additional sites were set up in Hawaii Volcanoes National Park on the island of Hawaii at locations where sawfly has been established since 1968 and sampled simultaneously using the same method.

Results and Discussion

The original release was made in April 1988 and establishment confirmed at all 5 release points within 3 months (Fig. 1). Within 1 yr, populations at the release points had increased substantially, and by 15 months, the larvae were found 1 km outside of the release area. A rapid population buildup and a quick dispersal of ft population over the entire infestation followed, and within 20 months a high and uniform population was encountered at all sites over the range.

Monitoring for the virus during the first 15 months was also negative; during this period no sign of the virus was found. Unfortunately at 19 months, at the peak of the population buildup, the virus suddenly appeared at most locations, and by the following sample period the population level had collapsed to a level apparently the same as that found at the three check areas on the island of Hawaii (which never exceeded 6 larvae found in 30 mins). Subsequent population sampling has shown that the population has remained at the same low level with a high frequency of virus.

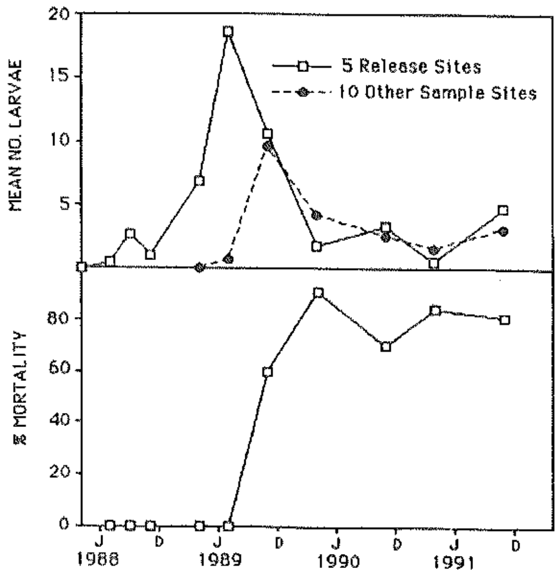

Figure 1. The upper graph shows the population of *Priophorus morio* larvae on the island of Kauai, Hawaii, following release in April 1988. The lower graph shows the percent of all larvae collected at each sampling period that died of a cytoplasmic polyhedrosis virus.

The very rapid buildup and visible feeding damage observed over the first 2 yrs (estimates were that by the fall of 1990, the sawfly population had destroyed between 10 and 15% of that year's foliage) indicate that *P. morio* was well on its way to becoming an effective biological control agent for *R. argutus*. The introduction of the virus has destroyed this potential on this island, but supports the theory that virus is the major factor limiting *P. morio* populations on the Hawaiian islands.

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