FINAL PROJECT REPORT

Project Title: DNA and morphometric diagnostics for apple and snowberry maggot flies

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Total Project Request: **Year 1:** $15,000 **Year 2:** $0 **Year 3:** $0

Other funding Sources: None

Total Project Funding: $15,000

Budget History

Item	**2009**		
Salaries	0		
Benefits	0		
Wages	13,600		
Benefits	1,400		
Equipment	0		
Supplies	0		
Travel	0		
Miscellaneous	0		
Total	**$15,000**		

RECAP ORIGINAL OBJECTIVES

Objectives:
1. Increase sample sizes for diagnostics using wing shape for apple and snowberry maggot flies.
2. Analyze ovipositor shape and male clasper shape to separate apple from snowberry maggot flies.

SIGNIFICANT FINDINGS

● By increasing the sample size, use of wing shape in an assignments test identified female apple maggot with 98.5% and female snowberry maggot with 99.0% accuracy,
● It correctly identified 100% of flies whose identities were questionable based on ovipositor lengths.
● Using canonical variates analysis (CVA), an assignments test using a CVA-distance based classification method, and multivariate analysis of variance, we found that clasper shape of flies from multiple sites accurately classified 99.8% of males to species.
● We found that ovipositor shape accurately classified 85.3% of females to species.
● Ovipositor length was longer in apple maggot than snowberry maggot, and combining ovipositor length with shape increased classification accuracy to 94.5%.
● Combining clasper or ovipositor shape and wing shape along with size of other structures in a single analysis should result in nearly 100% discrimination between species. This may benefit regulatory agencies and apple growers that depend on accurate identifications for fly quarantine and management measures.

RESULTS AND DISCUSSION

Wing Shapes of Female Flies. The wing of female apple maggot is more tapered at the tip than that of female snowberry maggot, giving it an overall narrower appearance than the wing of snowberry maggot, which is broader at the terminus (Figs. 1A and 1B). Canonical variates analysis (CVA) separated the wing shapes of six populations of female apple maggot and the two populations of snowberry maggot, although there was overlap between the two species (Fig. 2); the overlap was due to a few central WA apple maggots from black hawthorn (C Wash pom black haw).

There were misclassifications among conspecific populations of apple maggot, and some incorrect conspecific assignments between the two snowberry maggot populations. However, overall, 98.5% of female apple maggot and 99.0% of snowberry maggot were correctly identified to species. In addition, 100% of 18 'unknown' female apple maggots that fell in the ovipositor overlap zone were correctly identified as apple maggot using wing shape. They were assigned to five groups of apple maggot, but not to the C Wash pom black haw group. The one 'unknown' female snowberry maggot was also correctly identified using wing shape.

Wing Shapes of Male Flies. Bookstein coordinates indicate that the wing of male apple maggot is more tapered near the tip of the wing and is narrower than that of male snowberry maggot (not shown). As with females, CVA separated the wing shapes of the six populations of male apple maggot and the two populations of male snowberry maggot, and a few flies from the C Wash pom black haw group again fell between the two major species clusters (not shown). There were misclassifications within species, but 98.8% of apple maggot and 96.4% of snowberry maggot were correctly identified to species. We show for the first time that wing shape is a good character to discriminate between apple maggot and snowberry maggot in Washington state, even when the problematic central Washington black hawthorn flies are included.

Clasper Shape. The clasper configurations of apple maggot and snowberry maggot flies differed (Fig. 3). However, while the configuration in apple maggot is parallel, the configuration in snowberry maggot can be either divergent or parallel (Fig. 3B and 3C). Clasper shape of the species provided a more accurate reflection of species differences (Fig. 4). The clasper of apple maggot, as viewed from the side, is shovel or scoop shaped, with the upper curve slightly concave or flat and the bottom curve convex. The clasper of snowberry maggot is flipper or fin shaped, with upper curve distinctly sloping at the apex (SLMs 39–43 and LM 2) and the bottom curve only slightly convex

(Fig. 4). The CVA axes plot (Fig. 5) show little separation within species, but strong separation between them. Overall correct assignments at the species level were very high (Table 1). Addition of two measures of size, centroid size (CS) and length, did not appreciably improvement species discrimination. In sum, the results indicate that we obtained very good discrimination between species using clasper shape, and no statistically significant differences between populations of the same species.

Ovipositor Shape. The ovipositor shapes differ between species, as the ovipositor of apple maggot is proportionately more slender than that of snowberry maggot (Fig. 6). However, there was overlap in ovipositor shapes, as shown in the CVA plot (Fig. 7). Overall correct assignment to species was 85.3% using shape alone.

Ovipositor Length and Shape Combined. Apple maggot flies had longer ovipositors than snowberry maggot flies. When ovipositor length and shape were included in an assignments test, better separation was obtained than when shape alone was used (Table 2). Use of CS was less effective than use of length when combined with shape (Table 2).

Our findings are important for the Washington apple industry because they indicate that use of wing shape provides a significant improvement over the use of ovipositor length alone for identifying female apple maggot and snowberry maggot, thus potentially reducing misidentifications. Ovipositor shape is different between species, but there is overlap and it appears to be less useful than ovipositor length alone.

Our findings also show that wing shape and clasper shapes are different in males, and nearly 100% of individuals can be classified based on them. Clasper shape is more reliable than clasper configuration for species identification.

In practice, when ovipositor lengths and clasper configurations are ambiguous, wing or clasper shape analysis should be used, followed by use of multiple body measurements if ambiguity remains. A wing and clasper shape data base from known flies can be maintained by a regulatory agency such as WSDA and used to classify problematic flies (our data can be provided upon request). Morphometric programs for analysis and classification of unknowns used in this study are available online (http://www.canisius.edu/~sheets/ morphsoft.html). With training, it takes <30 min to photograph a wing or clasper of a fly, digitize it, subject it to analysis, and classify the fly. One caveat for using the method is that apple maggot in central WA, at least in our sample sites in wild areas from black hawthorn, may not be identified as accurately as flies from western WA using our current data.

In this project, shapes of different structures were analyzed separately. The possibility of combining wing, ovipositor, and clasper shape along with body measurements to discriminate 100% of flies should be investigated. If all flies are accurately identified, apple orchards would never mistakenly be placed under threatened or quarantine status and unmanaged apple or hawthorn trees would never need to be treated unnecessarily for apple maggot flies.

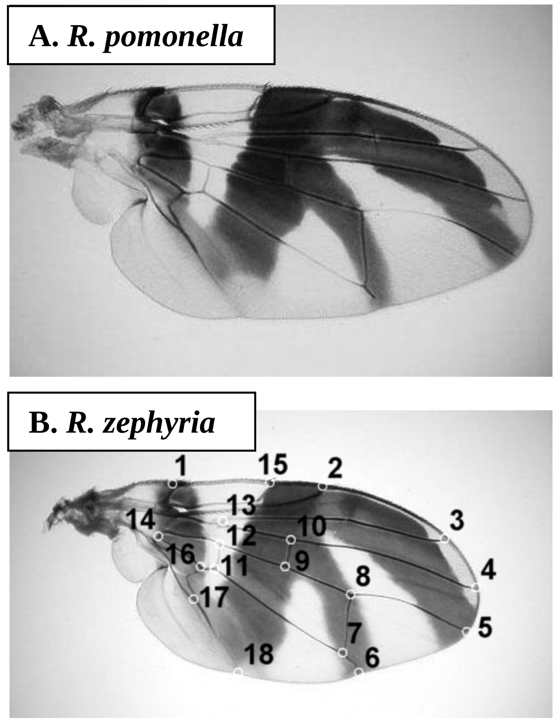

Fig. 1. Wings of (A) apple maggot and (B) snowberry maggot (with landmarks shown).

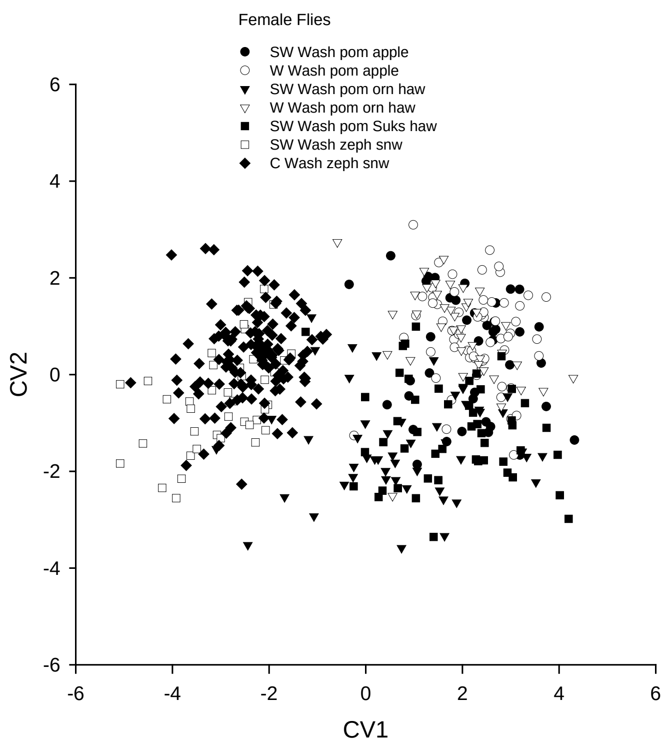

Fig. 2. Scatter plot from CVA of female apple maggot and snowberry maggot wings.

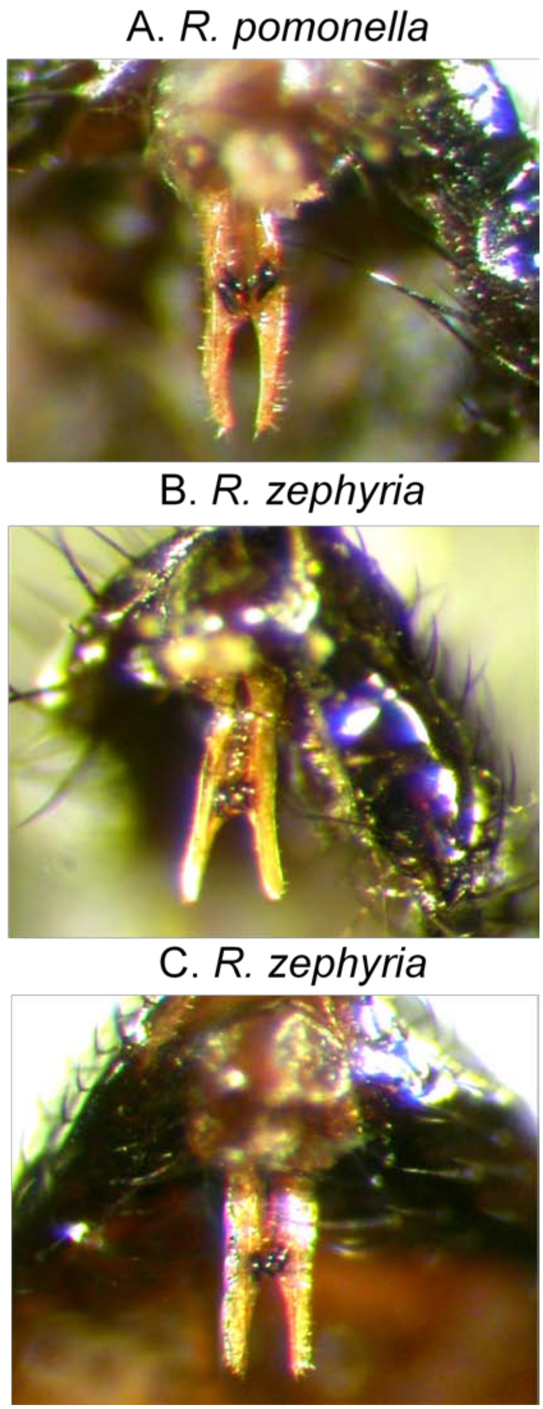

Fig. 3. Clasper configurations of (A) apple maggot and (B, C) snowberry maggot, showing variations in divergence.

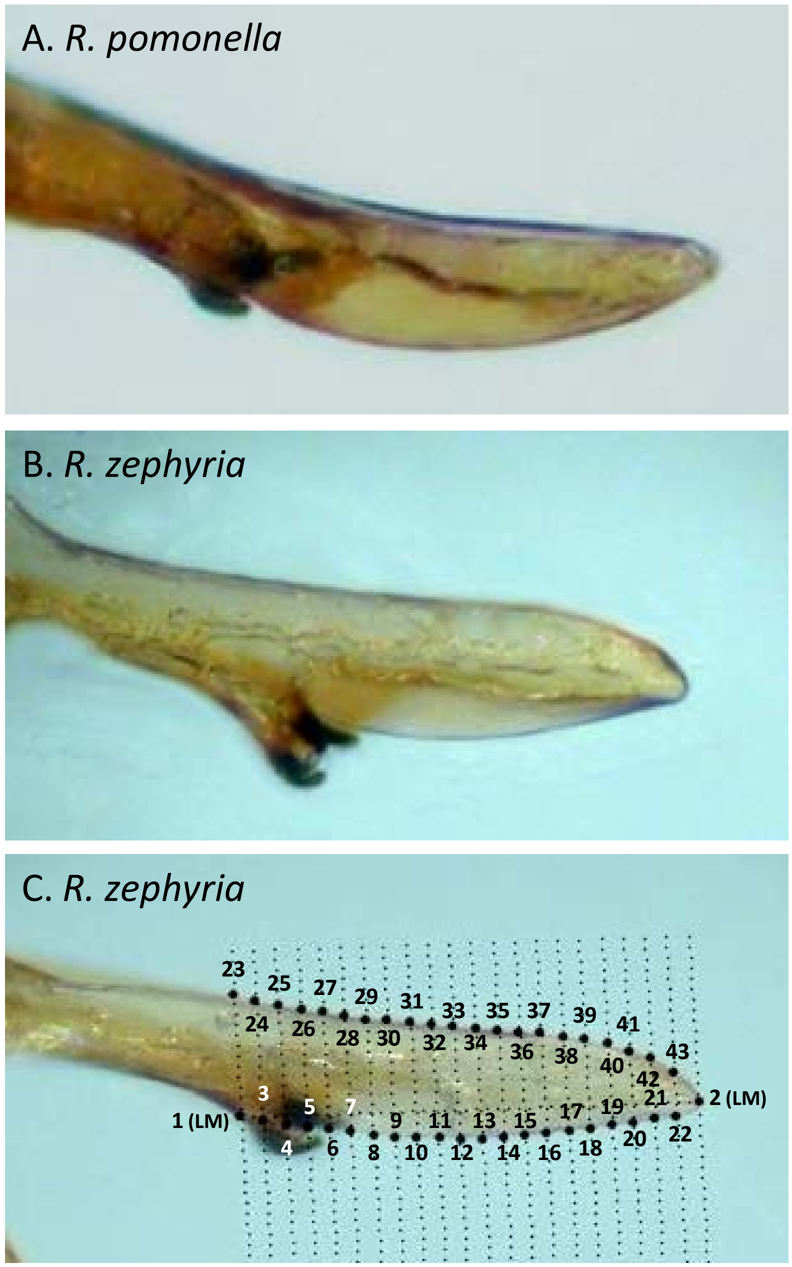

Fig. 4. Claspers of (A) apple maggot, (B) snowberry maggot, and (C) snowberry maggot showing landmarks and semi-landmarks.

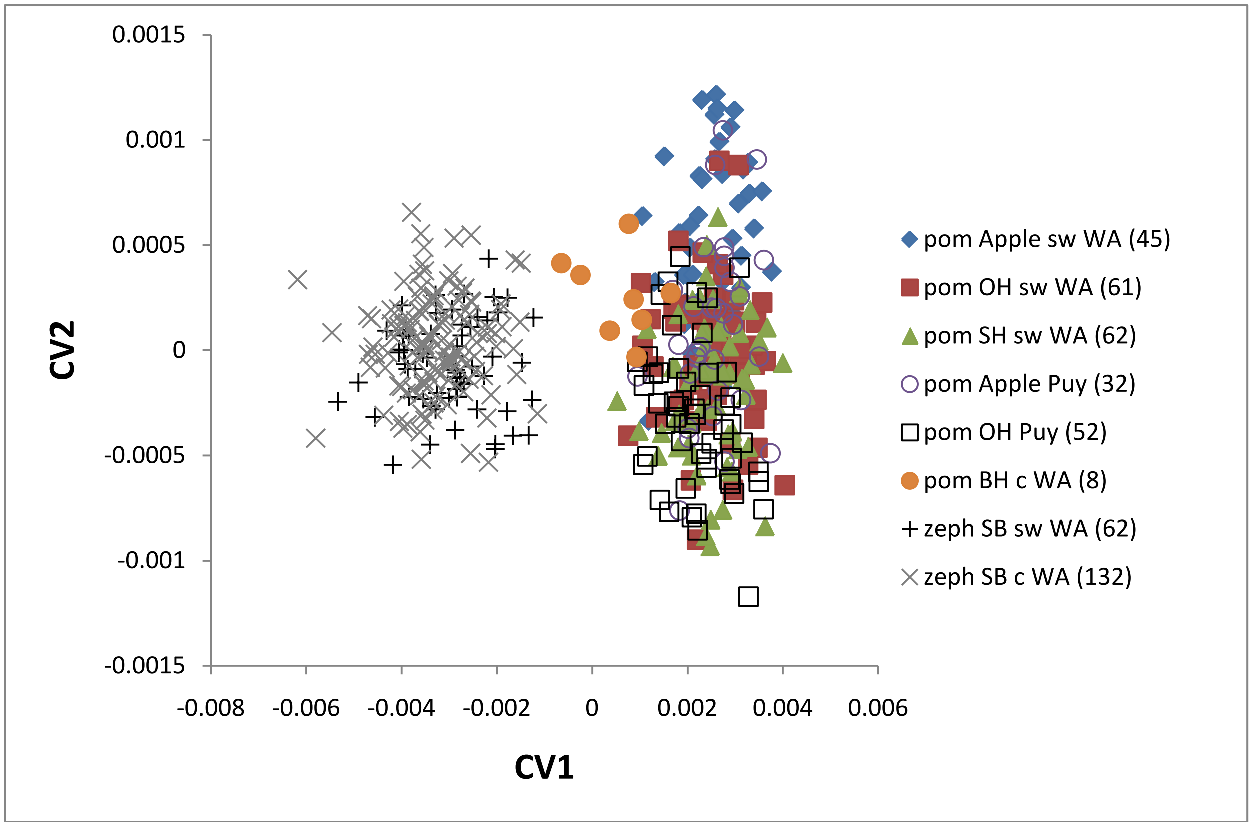

Fig. 5. Scatter plot from CVA of clasper shape of apple (pom) and snowberry maggot (zeph).

Table 1. Jackknifed groupings of 260 *R. pomonella* and 194 *R. zephyria* using surstylus shape and size from CVA-distance based method for assigning specimens to groups

Assignment Method	% correct, in 8 groups	% pom correct	% zeph correct	% species level correct
8 groups, shape only	41.2	100.0	95.9	98.2
2 groups, shape only	------	99.6	100.0	99.8
8 groups, shape + CS	40.3	100.0	96.9	98.7
2 groups, shape + CS	-----	99.6	100.0	99.8
8 groups, shape + length	40.3	100.0	96.9	98.7
2 groups, shape + length	-----	99.6	100.0	99.8

pom, *R. pomonella*; zeph, *R. zephyria*. CS, centroid size. 8 groups: six *R. pomonella* and two *R. zephyria*; 2 groups: all *R. pomonella* and all *R. zephyria*.

Table 2. Jackknifed groupings of 197 *R. pomonella* and 150 *R. zephyria* using aculeus shape and size from CVA-distance based method for assigning specimens to groups

Assignment Method	% correct, in 8 groups	% pom correct	% zeph correct	% species level correct
8 groups, shape only	37.2	90.4	78.7	85.3
2 groups, shape only	------	61.4	89.3	73.5
8 groups, shape + CS	43.2	86.3	94.7	89.9
2 groups, shape + CS	-----	71.1	97.3	82.4
8 groups, shape + length	43.2	94.4	94.7	94.5
2 groups, shape + length	-----	71.1	97.3	84.4

pom, *R. pomonella*; zeph, *R. zephyria*. CS, centroid size.
8 groups: six *R. pomonella* and two *R. zephyria*; 2 groups: all *R. pomonella* and all *R. zephyria*.

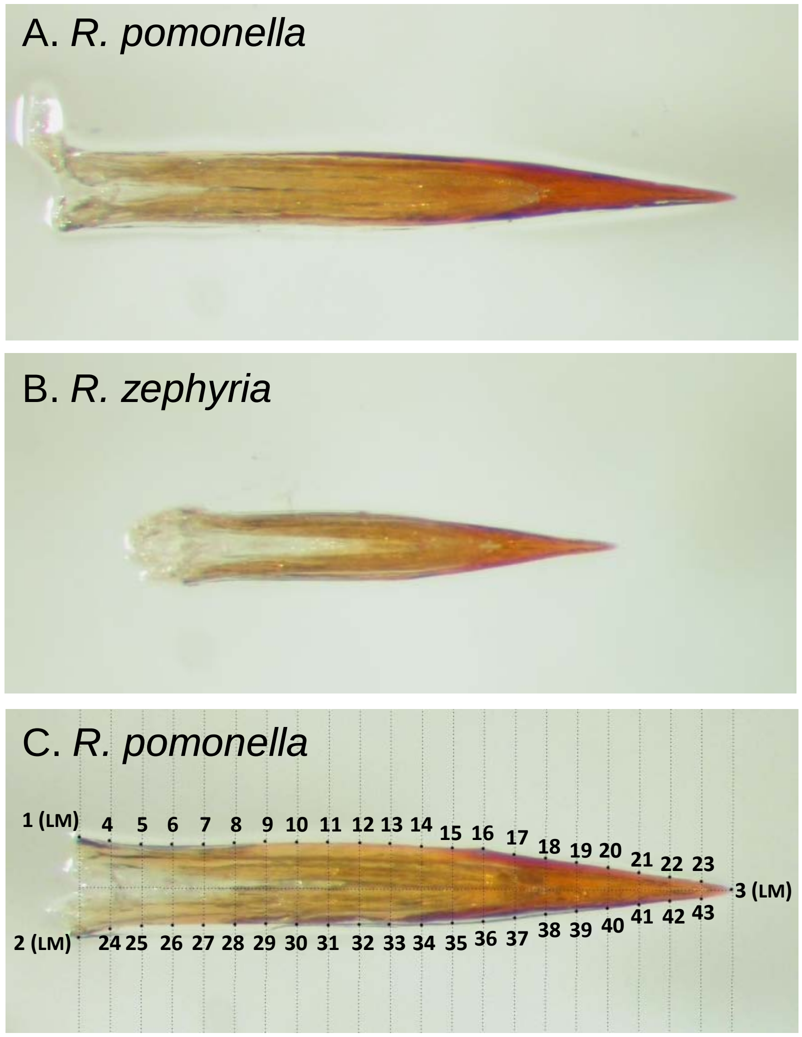

Fig. 6. Ovipositor of (A) apple maggot, (B) snowberry maggot, and (C) apple maggot, showing landmarks and semi-landmarks.

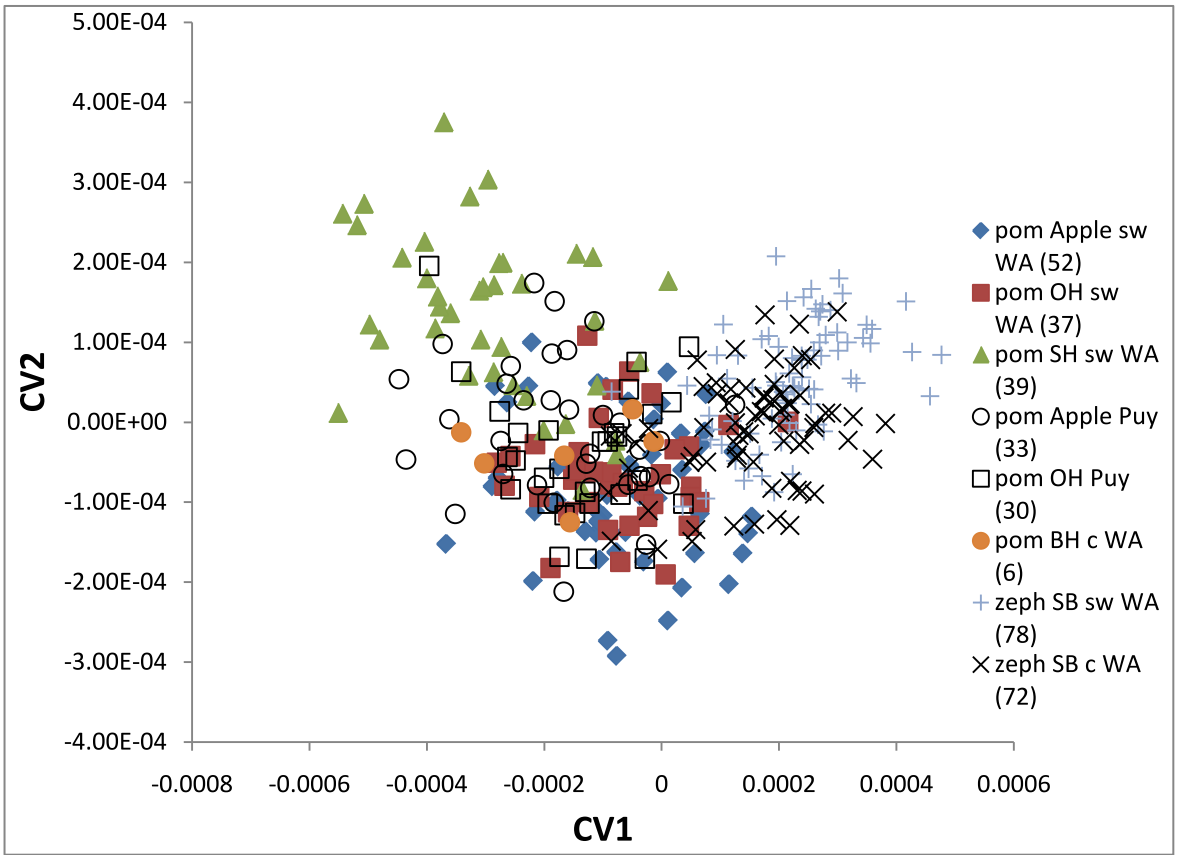

Fig. 7. Scatter plot from CVA of ovipositor shape of apple maggot (zeph) and snowberry (zeph) maggot.

EXECUTIVE SUMMARY

Apple maggot fly is a quarantine pest of apple in Washington state that is almost identical morphologically to snowberry maggot fly, a non-pest of apple. Historically, the longer ovipositor in apple maggot has been used to separate it from snowberry maggot, despite overlap in ovipositor lengths. Here, the objectives were to determine if use of wing shape, clasper shape, and ovipositor shape can be used to better discriminate of the species. Ovipositor lengths allowed 94.6% correct identification of female apple maggot but only 7.0% correct identification of snowberry maggot. Geometric morphometrics and CVA separated wing shapes between species in both sexes. Bookstein shape coordinates indicated that the wing of apple maggot is more tapered at the tip than that of snowberry maggot. Use of wing shape in an assignments test identified female apple maggot with 98.5% and female snowberry maggot with 99.0% accuracy, and it correctly identified 100% of flies whose identities were questionable based on ovipositor lengths. Results indicate that use of wing shape is an improvement over the use of ovipositor length alone for identifying female apple maggot and snowberry maggot in Washington state. Then we used geometric morphometrics to test the hypothesis that shapes of claspers and ovipositors of apple maggot and snowberry maggot differ. We found that all apple maggots had a nearly parallel surstyli configuration, but that many snowberry maggots had a parallel or divergent configuration. Using CVA, an assignments test using a CVA-distance based classification method, and multivariate analysis of variance, we found that clasper shape of flies from multiple sites accurately classified 99.8% of males to species. We found that ovipositor shape accurately classified 85.3% of females to species. Ovipositor length was longer in apple maggot than snowberry maggot, and combining ovipositor length with shape increased classification accuracy to 94.5%. Clasper and ovipositor shapes did not discriminate flies within species, regardless of host fruit and collection sites. Results suggest that use of clasper shape would benefit regulatory agencies and apple growers that depend on accurate identifications of apple maggot for quarantine and management measures. Combining clasper or ovipositor shape and shape and size of other structures in a single analysis should result in even greater discrimination (possibly 100%) between species. If all flies are accurately identified, apple orchards would never mistakenly be placed under threatened or quarantine status and unmanaged apple or hawthorn trees would never need to be treated unnecessarily for apple maggot.