Comparison of the Brown Sugar, Hot Water, and Salt Methods for Detecting Western Cherry Fruit Fly (Diptera: Tephritidae) Larvae in Sweet Cherry

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COMPARISON OF THE BROWN SUGAR, HOT WATER, AND SALT METHODS FOR DETECTING WESTERN CHERRY FRUIT FLY (DIPTERA: TEPHRITIDAE) LARVAE IN SWEET CHERRY

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ABSTRACT

Brown sugar and hot water methods have been developed to detect larvae of tephritid fruit flies post-harvest in fruit in order to maintain quarantine security. It would be useful to determine if modifications of these methods can yield better results and if less expensive alternatives exist. This study reports detection rates of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) larvae separated from crushed sweet cherries (*Prunus avium* [L.] L.) by brown sugar flotation versus hot water and sodium chloride (salt) flotation methods. Cherries were crushed and shredded by a standard cherry crusher and submerged either in brown sugar solution, hot water, or salt solution. In sugar and salt solutions, extracted larvae floated, whereas in hot water they sank; and in all 3 visual inspections for larvae were made. The brown sugar flotation method detected more total larvae than the hot water method when using a clear dish or black pan after cherries were shredded through a 2-mm gap in a cherry crusher, resulting in 95, 85, and 85% detections, respectively. Brown sugar and salt flotation methods resulted in similar detection rates of 85-99% after cherries were shredded through either a 2- or 5-mm gap, even though the 2-mm gap resulted in greater shredding. In brown sugar, hot water, and salt solutions, 26-88% of first instars (when there were at least 8 individuals) were detected versus 77-100% of second and third instars. Results demonstrate that salt and brown sugar solutions are equally efficacious for detecting larvae of *R. indifferens* separated from crushed cherries. Salt solution is advantageous over brown sugar solution because it is less expensive. Should salt solution be used for detecting larval spotted wing drosophila (*Drosophila suzukii* [Matsumura]) in cherries, current results show that it would not compromise detection of *R. indifferens*.

Key Words: western cherry fruit fly, brown sugar flotation, salt flotation, larval instars

RESUMEN

Se han desarrollado métodos utilizando el azúcar moreno y agua caliente para detectar larvas de moscas de la fruta tefritidas en frutas pos-cosechadas con el fin de mantener la seguridad de cuarentena. Sería útil para determinar si las modificaciones de estos métodos pueden producir mejores resultados y si existen alternativas de menos costo. Este estudio reporta las tasas de detección de larvas de *Rhagoletis indifferens* Curran (Diptera: Tephritidae) separadas de cerezas dulces aplastadas (*Prunus avium* [L.] L.) por medio de los métodos de flotación utilizando azúcar moreno y utilizando agua caliente y cloruro de sodio (sal). Las cerezas fueron aplastadas y trituradas por una trituradora de cereza estándar y fueron sumergidas en una solución de azúcar morena, agua caliente o solución salina. En soluciones de azúcar y sal, las larvas extraídas flotaban, mientras que en el agua caliente se hundian, por lo que un total de 3 inspecciones visuales fueron hechas para las larvas. El método de flotación de azúcar moreno detectó más larvas en total que el método de agua caliente cuando se utiliza un plato transparente u olla negra después de que las cerezas fueron trituradas a través de un hueco de 2 mm en una trituradora de cereza, que detectó el 95, 85 y 85 %, respectivamente. Los métodos de flotación utilizando azúcar moreno o sal resultaron en la detección de tasas similares del 85-99 % después de que las cerezas fueron triturados a través de un hueco de 2-5 mm, a pesar de que la brecha de 2 mm resultó en una mayor trituración. En las soluciones de azúcar morena, agua caliente y de sal, el 26-88 % de los primeros estadios (cuando había por lo menos 8 individuos) fueron detectados comparado con un 77-100 % para el segundo y tercer estadios. Los resultados demuestran que las soluciones de sal y del azúcar morena son igualmente eficaces para detectar larvas de *R. indifferens* separadas de cerezas trituradas. La solución salina es ventajosa con respecto a la solución de azúcar marrón, ya que es menos cara. En caso que la solución de sal sea utilizada para detectar larvas de la Drosophila con alas manchadas (*Drosophila suzukii*

[Matsumura]) en cerezas, los resultados actuales muestran que no pondría en peligro la detección de *R. indifferens*.

Palabras Clave: mosca de la fruta de cereza occidental, flotación con azúcar moreno, flotación con sal, estadios larvales

Variations of the brown sugar flotation and the hot water methods have been used to extract larvae of *Rhagoletis* fruit flies (Diptera: Tephritidae) from important fruit for many years. Hot water methods were used to extract eastern cherry fruit fly (*Rhagoletis cingulata* [Loew]) (Pettit 1926) and western cherry fruit fly (*Rhagoletis indifferens* Curran) (Shipman 1952; Frick 1953) from cherries. Hot water and brown sugar flotation methods were used to extract larval blueberry maggot (*Rhagoletis mendax* Curran) from blueberries (*Vaccinium* sp.) (Ericales: Ericaceae) (Lathrop & Nickels 1932; Neilson & Lawrence 1986; Neilson 1987; Dixon & Knowlton 1994). These methods were developed to detect larvae in fruit postharvest in order to maintain quarantine security and remain vital for fruit industries today (Brown 2009; Canadian Food Inspection Agency 2011). In addition, these methods can be used by experimenters for evaluating efficacies of insecticides for fly control or for determining infestation levels in fruit for biological studies. The methods are practical because they are simple to perform and require minimal equipment.

Rhagoletis indifferens is a quarantine pest of sweet cherry (*Prunus avium* [L.] L.; Rosales: Rosaceae) in the Pacific Northwest of the U. S., where fresh sweet cherries were valued at ~$509 million in 2012 (National Agricultural Statistics Service 2013). Most managed cherry orchards are free of the pest (Smith 2013), but there is zero tolerance for larval infestations in fruit destined for export (Anonymous 1968). To show the absence of larvae and achieve quarantine security, cherries in packing houses must be inspected. Brown sugar flotation and hot water methods are accepted by California for verifying the absence of larval *R. indifferens* in exported cherries as stated in California Department of Food and Agriculture (CDFA) Permit No. QC 783 (Brown 2009). In the brown sugar flotation and hot water methods, cherries are crushed to expose the larvae and then brown sugar solution or hot water is poured over the cherries, causing larvae either to float to the surface or sink to the bottom of containers, respectively (Yee 2012). The brown sugar flotation method detects greater percentages of larvae than the hot water method (Yee 2012), but as these are the only methods that have been evaluated, it would be useful to determine if variations or modifications of these methods can yield better results. Also, although brown sugar is not very expensive, other substances could be equally effective and less expensive than brown sugar, and thus more cost effective. Every season, 50-60 inspectors are employed in Yakima County, Washington (USA) alone by the Washington State Department of Agriculture to detect *R. indifferens* in cherries by the brown sugar method.

Several factors that could improve efficacies of the flotation and hot water methods have not been examined. One is the background color for the hot water method. In this regard the CDFA Permit No. QC783 (Brown 2009) states that a clear Pyrex dish or a dull black pan can be used. A clear Pyrex dish was used in Yee (2012), but a black pan was not; possibly the whitish larvae are more easily detected against a black than a clear background. Another factor is the degree of cherry shredding for the flotation method, defined here as the distribution of various large- and small-sized cherry pieces after crushing. Only one degree of cherry shredding was examined in Yee (2012); but perhaps greater shredding of cherries could liberate more larvae from fruit, allowing for greater detection efficiency.

Brown sugar has been the only substance used to create solutions for floating *R. indifferens* larvae. However, NaCl (sodium chloride) is more commonly used than sugar in solutions for floating other invertebrates: e.g, pyroglyphid mites (Arachnida: Acari: Pyroglyphididae) from house dust and rearing medium (Sasa et al. 1970; Hart & Fain 1987); ceratopogonid midge larvae (Diptera: Ceratopogoniade) from mud (Rees & Winget 1970; Rees et al. 1971; Mullens & Rodriguez 1984); and curculionid and chrysomelid beetles from grains (Richter & Tchalale 1994). Past researchers used sodium chloride because it is less expensive than sugar (Mullens & Rodriguez 1984; Hribar 1990). Surprisingly, sodium chloride has never been evaluated for detecting tephritid larvae from fruit, although recently it was mentioned in passing for detecting European cherry fruit fly (*Rhagoletis cerasi* L.) larvae in cherries (Dederichs 2013). Sodium chloride solution could irritate larvae more than sugar, and cause more of them to move and leave the crushed fruit; and thus allow more larvae to be detected.

Evaluating the use of salt solutions is also important because of the presence of the spotted wing drosophila (*Drosophila suzukii* [Matsumura]) (Diptera: Drosophilidae) in cherry-growing areas. This recent pest in the U.S.A. can also infest cherry orchards (Beers et al. 2011) and also the possible presence of its larvae in cherries needs to be determined. Sodium chloride solution is recommended over sugar solution for extract-

ing larvae of *D. suzukii* from whole blueberries because it is less expensive and less solute is needed to make a useful solution (Hueppelsheuser 2010; Bruck et al. 2011).

The main objective of this study was to compare brown sugar against hot water and sodium chloride solutions for detecting *R. indifferens* larvae separated from crushed cherries. The hypothesis was tested that salt solutions is as efficacious as brown sugar solution. Another objective was to compare the effects of crushing cherries through 2 crusher gap sizes that produce different degrees of cherry shredding on larval detection rates. The hypothesis was tested that the smaller gap size results in greater larval detection.

Materials and Methods

The Brown Sugar Method versus the Hot Water Method

In 2010, 'Bing' sweet cherries were collected from 5 trees at the USDA-ARS Research Farm ~16 km east of Moxee, Washington State (WA), U. S., on 7, 12, and 14 Jul and inspected for larvae on 8, 13, and 15 Jul, respectively. Cherries were held at 3-5 °C for ~24 h before crushing. The diam and Brix (measured by a refractometer, Atago N1, Japan) of 10 cherries from each tree were measured. Larvae in cherries from each of the 5 trees were extracted by 3 methods: (1) 14.4% brown sugar (w:w) flotation, (2) hot water submersion in a clear Pyrex dish, and (3) hot water submersion in a black pan. 'Control' or uncrushed cherries were initially included for comparison, but ultimately were not used in this study because there was no way to generate control larval detection rates when cherries were not crushed. Each of 3 inspectors crushed a 454-g cherry sample from each tree for each method. Methods were as described in Yee (2011) with 2 main differences: (1) the crusher gap used here was 2 mm instead of 5 mm in order to more thoroughly shred the cherries (see Yee [2012] for a description of the crusher); and (2) a black pan was used (see below). For all 3 methods, crushed cherries fell in a tub (30.5 cm long × 25 cm wide × 14 cm high). The brown sugar (Amalgamated Sugar Co. LLC, Boise, Idaho) solution was made using 3.18 kg sugar per 18.9 L of water (14-16% sugar by wt or vol), i.e., a density of 1.051 mg per mL. Two L of solution were poured onto the crushed cherries in the tub to submerge them. Anti-foamer (10% dimethylpolysiloxane, Genesis Agri Products Inc., Yakima, Washington) (2 mL per 15 L) was added to the solution. A refractometer was used to ensure the mixture of brown sugar solution and juice from crushed cherries remained at 14.5-15.5% Brix. The crushed cherries in solution were allowed to settle for 5 min; then the surface of the solution was examined for 6 (8 Jul) or 7 min (13 and 15 Jul, due to more larvae). Larvae that floated to the surface were collected and preserved in 70% ethanol for later counting. Larvae recovered at this and all evaluation steps below were measured: first instars were 0-2.0 mm in length, second instars 2.1-4.5 mm, and third instars 4.6-8 mm (Frick et al. 1954). For hot water submersion methods, a Pyrex dish (39.5 cm long × 27 cm wide × 6 cm high) or a black pan (black polypropylene Bussing Box, SKcamel, Korea; 52 cm long × 39 cm wide × 12.5 cm high) was used. For both, crushed cherries fell from the crusher onto a liner of metal hardware cloth (0.635 cm square openings) in a tub. Two L of hot water (60-82 °C) were poured onto the crushed cherries to submerge them; after 1 min, the liner with crushed cherries was removed, leaving the hot water in the tub. The hot water was then poured into the Pyrex dish or the black pan. Light from below was used to illuminate larvae in the Pyrex dish, and light from above in the black pan, to give a light intensity of ~0.215 × 1000 foot-candles near the larvae. Larvae were collected over 6 or 7 min and preserved in 70% ethanol for later counting and measuring. Inspections for larvae were conducted outdoors in the shade under a roof edge between 0900 and 1400 hours on the 3 days, which were sunny or partly sunny.

Efficacy of the 3 methods was evaluated by the procedures in Yee (2012). To determine numbers of larvae not detected by the brown sugar method during the 6- or 7-min inspections, crushed cherries and the solution in the tub were poured through successive sieves (screens) with 600, 250, and 125 µm openings (USA Standard test sieve, Hogentogler & Co., Inc., Columbia, Maryland) to filter out cherry pieces (on the 600 µm sieve) and any larvae (on all sieves). Crushed cherry pieces were removed from the sieve and transferred to plastic containers with 70% ethanol. Each screen was then inverted and sprayed with water into a clean tub. The water in the tub was poured into a container for storage and refrigerated. Within a week, the water was then poured onto a coffee filter and the filter inspected for larvae under a stereomicroscope. The 2 hot water methods were similarly evaluated, except crushed cherries were on a liner and were directly transferred to 70% ethanol without needing to be poured onto sieves.

Each cherry piece preserved in 70% ethanol was examined for larvae under a microscope. The degree of shredding was the distribution of percentages of cherry pieces in 7 categories: whole, seed (pyrene) present (+); whole, not split; whole, seed absent (-); half, seed present (+); half, seed absent (-); partial, seed present (+); and partial, seed absent (-), as shown in Yee (2011).

Brown Sugar Solution versus Salt Solution at Two Crusher Gap Widths

In 2011, 'Bing' sweet cherries were collected from 4, 5, and 6 trees at the USDA-ARS Research

Farm on 17, 18, and 19 Jul and inspected for larvae on 18, 19, and 20 Jul, respectively. Cherries were held at 3-5 °C for ~24 h before crushing. Diameters, weights and Brix of 10 cherries from each tree were measured. The firmness of cherries was also measured by a push-pull dynamometer penetrometer with a 1-mm diameter tip (Facchini, Alfonsine, Italy).

Comparisons between 2-mm and 5-mm gap widths were made for the brown sugar and the sodium chloride (= salt) flotation methods: (1) 14.4% brown sugar (w:w), 2-mm gap, (2) 7.5% salt (w:w), 2-mm gap, (3) 14.4% brown sugar, 5-mm gap, and (4) 7.5% salt, 5-mm gap. Three inspectors each crushed 454-g cherry samples from each tree for each of the 4 methods. General detection and evaluation procedures for both brown sugar and salt methods were the same as for the brown sugar method in 2010. Preliminary tests had compared 5%, 10%, and 15% salt solutions; as both 5% and 10% floated all of the larvae, a 7.5% solution was chosen. Salt (Morton® table salt, Morton International, Inc., Chicago, Illinois) solution was made using 1.47 kg salt per 18.2 L of water, generating a density of 1.055 mg per mL. Two L of salt solution were poured onto the crushed cherries. To darken the salt solution so that larvae could be seen more easily, caramel color (McCormick & Co., Hunt Valley, Maryland) was added (19 mL per 18.9 L of solution). Anti-foamer was added to both brown sugar and salt solutions (2 mL per 15 L). The Brix of the brown sugar or salt solution mixed with juice from crushed cherries was measured with a refractometer to ensure that it remained at 14.5-15.5%. Crushed cherries were allowed to soak for 5 min; solutions were then examined for larvae for an additional 5 min. Larval inspections were conducted in the shade outdoors as in 2010; evaluation of all 4 methods was the same as for the brown sugar method in 2010.

Statistical Analysis

To compare percentages of larvae recovered, comparisons of multiple proportions (χ^2) or of 2 proportions (Z_c, when only 2 instar groups had sufficient numbers) were conducted (Zar 1999). When there were significant differences in > 2 group comparisons, Tukey-type multiple comparisons of proportions were conducted (Zar 1999). Data from the 3 inspectors were combined to generate larger larval numbers for the analyses.

Results

The Brown Sugar Method versus the Hot Water Method

Cherries were 24-25 mm in diam with Brix readings of 17-22%. On all 3 dates, the brown sugar method was more effective than both hot water methods for detecting total larvae (Table 1), due mostly to higher detections of first instars in the brown sugar solution. Within all methods, first instars were detected at lower rates than second and third instars. On 15 Jul only, lower percentages of second than third instars were detected (Table 1). The extent of shredding of cherries in the brown sugar and in one or both hot water methods differed as indicated by 3 (whole, seed+; whole, seed-; partial, seed-) of 7 categories. However, percentages of half pieces and of partial pieces with seeds (pyrenes) among the methods did not differ (Table 2). Because only 3 categories differed among the various methods, it is unlikely the extent of shredding was a major factor contributing to differences in detection among them.

Brown Sugar versus Salt Solutions at 2 Crusher Gap Widths

Cherries were 27-28 mm in diameter, had firmness (penetration resistance) of 19-20 kg per cm^2, weighed 9-10 g, and had Brix values of 17-18%. On all 3 dates with respect to the second and third instars, none of the 4 methods differed statistically (Table 3). Too few (0-13) first instars were present within the dates for analyses across the different methods. To generate at least 20 first instars within brown sugar and within salt treatments (both gap widths), data from all dates were combined. When this was done, no significant difference in percentages of first instars detected in the brown sugar and salt solutions was detected (58.1%, n = 43, versus 34.8%, n = 23) (Z_c = 1.548; $P > 0.05$). However, for both sugar and salt solutions (all dates combined, both gap widths), lower percentages of first instar than second and third instars were detected (sugar: Z_c = 8.639; $P < 0.05$; salt: Z_c = 11.312; $P < 0.05$). On all 3 dates with respect to the brown sugar methods, crushing through the 2-mm gap resulted in 2-3% greater overall larval detection than the 5-mm gap, but these differences were not significant ($P > 0.05$). Relative percentages for 5 of 7 cherry piece categories showed that the 2-mm gap more thoroughly shredded the cherries than the 5-mm gap (Table 4).

Discussion

A summary of findings is as follows: (1) The brown sugar flotation method is more effective than the hot water method with either a clear dish or black pan for detecting larvae of *R. indifferens* from crushed sweet cherries; (2) flotation by the salt solution is as effective as by the brown sugar solution, and this supports the first hypothesis; (3) greater cherry shredding caused by 2-mm than by the 5-mm crusher gap width did not result in greater larval detections in either the brown sugar or the salt solution, so the second

TABLE 1. PERCENTAGES OF *RHAGOLETIS INDIFFERENS* LARVAE DETECTED FROM CRUSHED CHERRIES BY THE BROWN SUGAR FLOTATION AND HOT WATER (HW) DETECTION METHODS ON 3 DATES IN 2010. TOTAL NUMBERS OF LARVAE INSIDE PARENTHESES.

8 Jul (6-min inspection, from 5 total tree samples)					
Larval Instar	Brown Sugar	HW, Clear Dish	HW, Black Pan	χ^{2a}	*P*
First	69.8 (43)Ab	29.8 (57)Bb	37.1 (62)Bb	17.452	*****
Second	98.6 (140)Aa	93.5 (138)Aa	93.0 (158)Aa	5.712	NS
Third	100 (88)Aa	98.1 (105)Aa	96.6 (88)Aa	2.908	NS
χ^{2b}	60.356	141.957	114.384	—	—
P	*****	*****	*****	—	—
All instars	94.5 (271)A	83.0 (300)B	82.8 (308)B	21.501	**
13 Jul (7-min inspection, from 5 total tree samples)					
Larval Instar	Brown Sugar	HW, Clear Dish	HW, Black Pan	χ^{2a}	*P*
First	67.5 (40)Ab	26.7 (45)Bc	48.1 (52)ABc	14.257	****
Second	97.1 (138)Aa	83.3 (138)Bb	85.8 (134)Bb	14.906	****
Third	99.4 (322)Aa	96.9 (322)Aa	97.1 (342)Aa	5.792	NS
χ^{2b}	99.357	173.204	116.772	—	—
P	*****	*****	*****	—	—
All instars	96.2 (500)A	86.9 (505)B	89.4 (528)B	27.754	**
15 Jul (7-min inspection, from 5 total tree samples)					
Larval Instar	Brown sugar	HW, Clear dish	HW, Black pan	χ^{2a}	*P*
First	62.9 (35)Ab	25.8 (31)Bc	25.7 (35)Bc	13.287	****
Second	97.0 (66)Aa	77.2 (57)Bb	85.0 (60)Bb	10.790	**
Third	96.4 (280)Aa	95.4 (261)Aa	95.7 (277)Aa	0.392	NS
χ^{2b}	58.435	117.434	138.578	—	—
P	*****	*****	*****	—	—
All instars	93.4 (381)A	86.2 (349)B	87.4 (372)B	11.463	***

[a]Within-row comparisons.
[b]Within-column comparisons.
Per date: combined numbers from 3 inspectors, 5 trees, and 6.8 total kg of cherries per method. *****, $P < 0.001$; ****, $P < 0.005$; ***, $P < 0.01$; **, $P < 0.025$; *, $P < 0.05$; NS, $P > 0.05$.
Percentages followed by the same uppercase letters within rows and same lowercase letters within columns are not significantly different (multiple comparisons for proportions, $P > 0.05$).

hypothesis was not supported. The finding that overall larval detection rates were >90% show the high efficacies of flotation by the brown sugar and salt solutions, compared with recovery rates of other invertebrates from various substrates, which range widely from 14-100%. No extraction method for arthropods appears to consistently recover 100% of individuals (Anderson 1959; Fast 1970; Pask & Costa 1971; Mullens & Rodriguez 1984; Hribar 1990; André & Noti 1993; Dixon & Knowlton 1994; André et al. 2002).

Results indicate that by the hot water method, the whitish *R. indifferens* larvae were equally difficult to see against either a black or clear background. Whether a Pyrex dish or a black pan was used, the turbid mix of water and cherry juice under artificial and natural daylight reduced the visibility of the larvae. This was especially true of the first instars, which are small and can appear similar to fragments of cherry debris and can also be hidden beneath them. The lower detections of first than second and third instars by the hot water and brown sugar methods are consistent with detections of *Rhagoletis mendax* separated from blueberries. No first instars of *R. mendax* were obtained from crushed blueberries by the brown sugar flotation method, whereas 21% of all larvae detected through dissection of blueberries were first instars (Dixon & Knowlton 1994). Given the hot water method's weaker performance than the brown sugar method here and in a previous study (Yee 2012), the hot water method for detecting *R. indifferens* larvae, using either a Pyrex dish or black pan, should be removed from the CDFA permit, unless additional steps to improve the method are identified.

Use of a hot water method for detecting *R. mendax* in uncrushed blueberries apparently was more successful than that reported here for *R. indifferens* in crushed cherries. Neilson (1987)

TABLE 2. PERCENTAGES OF CHERRY PIECES IN DIFFERENT CATEGORIES AFTER CRUSHING AND PROCESSED THROUGH THE BROWN SUGAR AND HOT WATER (HW) *RHAGOLETIS* DETECTION METHODS. COMBINED DATA FROM 8, 13 AND 15 JULY 2010.

	Percentage Pieces Out of Total				
Cherry Category	Brown Sugar	HW, Clear Dish	HW, Black Pan	χ^2	*P*
Whole, Seed+	9.85 B	12.45 A	2.47 A	22.122	*****
Whole, Not Split	0	0	0	—	—
Whole, Seed–	6.14 B	8.19 A	6.73 B	16.456	*****
Half, Seed +	14.81	15.95	15.79	2.869	NS
Half, Seed –	28.64	28.39	27.95	0.602	NS
Partial[a], Seed+	6.51	7.50	6.85	3.787	NS
Partial[a], Seed –	34.05 A	27.52 C	30.21 B	49.384	*****
Total Pieces	5,098	4,628	4,877		

[a]<half a cherry.

*****, $P < 0.001$; ****, $P < 0.005$; ***, $P < 0.01$; **, $P < 0.025$; *, $P < 0.05$; NS, $P > 0.05$.

Percentages followed by the same letters within rows are not significantly different (multiple comparisons for proportions, $P > 0.05$).

concluded that either the hot water or the brown sugar flotation method could be used for blueberries, but that the flotation method was faster. The higher efficiency of the hot water method for detecting *R. mendax* in blueberries than for *R. indifferens* in cherries has several possible explanations. Crushed cherries were not sprayed with water; the amount of blueberries crushed was small, allowing most larvae to be seen; spraying with water improved the clarity of the solution such that *R. mendax* larvae stood out against their surroundings; and the examination time of blueberries in hot water was twice as long as that of cherries in the current study.

Percentages of *R. indifferens* larvae detected in 14.4% brown sugar solution were comparable to those of *R. mendax* larvae recovered from crushed blueberries in 17.5% brown sugar, which were 87.8-95.8% (Neilson & Lawrence 1986; Neilson 1987). This was based on counting larvae that floated and by pouring crushed blueberries onto a screen and spraying with water until no larvae were recovered. Surprisingly, 80.2% and 77.9% of *R. mendax* larvae floated to the surface of 15.0% and 20.0% brown sugar solution, respectively, versus 91.1% in 17.5% solution (Neilson 1987), suggesting some optimal density for floating larvae could exist.

The results did not provide evidence that salt irritates *R. indifferens* larvae more than sugar, causing more of them to leave crushed fruit. Over the 11-12 total min that larvae were immersed in the solution, the 7.5% salt would be expected to cause some cell shrinkage leading to excessive movements (in general, equivalents of 0.9 to 1.5% sodium chloride cover the range of osmotic pressures in insect haemolymph [Nation 2002]). However, if cell shrinkage caused excessive larval movements to occur, it did not result in greater detections. The densities of salt and brown sugar solutions were similar (1.055 versus 1.051 g per mL) and when solutions were mixed with juice from crushed cherries the Brix values were maintained similarly at 14.5-15.5%, which apparently allowed larvae to float equally in the 2 solutions.

Salt solution is recommended for extracting *D. suzukii* from whole blueberries based on the premise that it irritates larvae and causes them to wiggle out of the holes in fruit (Bruck et al. 2011). However, observations show that 5.9% salt solution floated only about 63% of *D. suzukii* larvae whereas 14.9% sugar floated at least 90%. However, the sugar solution also floated all of the uncrushed blueberries, impeding larval counts, whereas salt solution did not, making salt solution more useful (Hueppelsheuser 2010). These and current results indicate that the choice of using sugar or salt for larval detection depends on the type and condition of fruit being inspected as well as the larval species. Uncrushed cherries also floated in salt and brown sugar solutions but crushed cherries did not, probably because crushing removed air spaces and increased their densities (WLY, unpublished observations).

The lack of overall differences in detection levels between the brown sugar and the salt flotation methods suggests that sodium chloride solution could be an option to replace brown sugar solution for detecting *R. indifferens* larvae. The 2 methods also are similar in that both failed to detect first instars at the same rate as the larger second and third instars. The main advantage of using salt, then, is lower cost. In 2012, Washington State Department of Agriculture (Yakima, Washington) paid US $18.79 per 11.34-kg bag of brown sugar; 300 bags were purchased for a total of $6,576.50, charged to the packing houses. This did not include sugar bought by packing houses themselves. An 11.34-kg bag of plain table salt cost $5.26 in 2013 or 3.6 times less than brown sugar.

TABLE 3. PERCENTAGES OF *RHAGOLETIS INDIFFERENS* LARVAE DETECTED IN SWEET CHERRIES CRUSHED AT 2- AND 5-MM CRUSHER GAP WIDTHS AND SEPARATED BY THE BROWN SUGAR (BS) AND SALT FLOTATION METHODS. TOTAL NUMBERS OF LARVAE SHOWN WITHIN PARENTHESES.

18 Jul (5-min inspection, from 4 total tree samples)						
Larval Instar	BS, 2 mm	Salt, 2 mm	BS, 5 mm	Salt, 5 mm	χ^{2a}	*P*
First	0 (4)	0 (3)	0 (4)	- (0)	—	—
Second	- (0)	0 (4)	100 (4)	100 (1)	—	—
Third	100 (46)	100 (40)	100 (30)	97.3 (37)	2.953	NS
All instars	92.0 (50)	85.1 (47)	89.5 (38)	97.4 (38)	3.873	NS
19 Jul (5-min inspection, from 5 total tree samples)						
Larval Instar	BS, 2 mm	Salt, 2 mm	BS, 5 mm	Salt, 5 mm	χ^{2a}	*P*
First	87.5 (8)	44.4 (9)	69.2 (13)	50.0 (8)	—	—
Second	91.5 (47)	97.8 (45)	97.1 (35)	93.2 (59)	2.442	NS
Third	87.3 (63)	93.5 (46)	84.3 (51)	89.1 (55)	2.087	NS
Z_c[b]	0.390	0.491	15.441	0.117		
P	NS	NS	*	NS		
All instars	89.0 (118)	91.0 (100)	86.9 (99)	88.5 (122)	0.871	NS
20 Jul (5-min inspection, from 6 total tree samples)						
Larval Instar	BS, 2 mm	Salt, 2 mm	BS, 5 mm	Salt, 5 mm	χ^{2a}	*P*
First	83.3 (6)	0 (2)	50.0 (8)	0 (1)	—	—
Second	98.1 (52)	97.8 (45)	95.5 (67)	98.6 (74)	1.560	NS
Third	100 (67)	97.6 (84)	100 (74)	100 (78)	3.962	NS
Z_c[b]	0.015	- 0.548	1.260	0.023		
P	NS	NS	NS	NS		
All instars	98.4 (125)	96.2 (131)	95.3 (149)	98.7 (153)	4.117	NS

[a]Within-row comparisons.
[b]Within-column comparisons; test of 2 proportions (Z_c), only 2nd versus 3rd instars; excludes 1st instar.
Per date: combined numbers from 3 inspectors, 4 to 6 trees, and 5.4–8.2 total kg of cherries per method.
*****, $P < 0.001$; ****, $P < 0.005$; ***, $P < 0.01$; **, $P < 0.025$; *, $P < 0.05$; NS, $P > 0.05$. Percentages followed by the same uppercase letters within rows are not significantly different (multiple comparisons for proportions, $P > 0.05$).

In addition, less salt is needed for effectively detecting *R. indifferens*. A 7.5% salt solution is as effective as a 14.4% brown sugar solution, requiring only 70 g of salt versus 168 g of brown sugar in 1 L of water, further decreasing the cost of using salt. Thus, not only is salt 3.6 times cheaper, but only ~half as much is needed, so it is actually ~7.2 times cheaper to use. A 473-mL bottle of caramel cost only $7.37 in 2011, and is enough to darken 473 L of 7.5% salt solution. Because of the small amounts of salt used in solution, the disposal of salt solution is not likely to be a problem. $MgSO_4$ (Mullens & Rodriguez 1984) and CCl_4 (André & Noti 1993) are other possible substances ($MgSO_4$ is also much less expensive than brown sugar) that can be used for flotation but have yet to be tested against *R. indifferens*.

Results did not support the hypothesis that greater shredding of cherries could liberate more larvae from fruit, allowing for greater detections. Over 3 collections, detections from the brown sugar method averaged 2.6% higher by the 2-mm than by the 5-mm gap width. Given the already high (~90%) detection rates, any percent improvement would be expected to be small. Whether the numerical difference translates into greater detections when larval populations are very low, as might be expected in infested commercial orchards, needs further examination. However, the lack of numerical differences between gap widths using the salt solution suggests that higher degrees of shredding caused by the 2-mm gap are not needed to liberate more larvae.

The brown sugar method is accepted by California (Brown 2009) for detecting *R. indifferens*. Given the effort it may take to change permit regulations, it is not suggested that the salt method replace it immediately, but that an effective option exists for replacing the hot water method. Also, the salt solution method could be useful for non-regulatory personnel. For example, small-scale orchardists or homeowners who do not export fruit and who want to know immediately if their cherries are infested with *R. indifferens* could

TABLE 4. PERCENTAGES OF SWEET CHERRY PIECES IN DIFFERENT CATEGORIES AFTER CRUSHING AT 2- AND 5-MM CRUSHER GAP WIDTHS AND SEPARATED BY THE BROWN SUGAR (BS) AND SALT FLOTATION METHODS. COMBINED DATA FROM 18, 19 AND 20 JULY 2011.

	Percentage Pieces out of Total					
Category	BS, 2 mm	Salt, 2 mm	BS, 5 mm	Salt, 5 mm	χ^2	*P*
Whole, Seed+	4.45 B	3.62 B	9.79 A	9.93 A	283.762	*****
Whole, Not Split	0.09	0.04	0.12	0.10	2.377	NS
Whole, Seed–	2.59 A	2.58 A	3.38 A	2.54 A	9.294[b]	*
Half, Seed +	11.42 B	10.94 B	14.73 A	13.87 A	48.673	*****
Half, Seed –	16.64	17.16	16.34	15.34	6.679	NS
Partial[a], Seed+	10.32 AB	11.00 A	9.15 B	9.23 B	14.142	****
Partial[a], Seed –	54.49 A	54.66 A	46.49 B	48.99 B	104.808	*****
Total Pieces	5,553	5,467	5,036	5,046		

[a]< half a cherry.

*****, $P < 0.001$; ****, $P < 0.005$; ***, $P < 0.01$; **, $P < 0.025$; *, $P < 0.05$; NS, $P > 0.05$. Percentages followed by the same letters within rows are not significantly different (multiple comparisons for proportions, $P > 0.05$).

[b]Multiple comparisons were not significant ($P > 0.05$).

find that salt is a better choice than brown sugar because of lower cost and amount needed. Similarly, experimenters who want to quickly compare larval infestations in different insecticide treatments could use the salt solution method. A food/potato masher with square openings (Neilson & Lawrence 1986; Neilson 1987; Dixon & Knowlton 1994) possibly could be used to crush cherries if a crusher is not available. Alternatives to flotation methods for determining larval infestation rates in fruit are rearing larvae, which can take 3-4 weeks, and dissecting fruit manually (Frick 1953; Dixon & Knowlton 1994); these methods are not desirable when determinations must be made rapidly.

In conclusion, results demonstrate that salt and brown sugar solutions are equally efficacious for detecting larvae of *R. indifferens* from crushed cherries. Salt solution is advantageous over the brown sugar solution because it is less expensive. Results suggest either brown sugar or salt method could be used to detect larvae at packing houses to fulfill cherry export requirements and for experimental work where quick determinations of infestation rates are desired, although both methods could underestimate the frequencies of first instars. Should salt solution be used for detecting larval *D. suzukii* in cherries, the current results show that it will not compromise the detection of *R. indifferens*.

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