Detection/Monitoring of *Bactrocera latifrons* (Diptera: Tephritidae): Assessing the Potential of Prospective New Lures

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Abstract. *Bactrocera latifrons* is a tephritid fruit fly (Diptera: Tephritidae) which has a host list of 59 plant species from 14 plant families, with over 70% of the host plant species coming from the plant families Solanaceae and Cucurbitaceae. *Bactrocera latifrons* is of primarily Asian distribution, but its range has expanded through introductions into Hawaii, Okinawa (Japan), Tanzania, and Kenya. The documented introductions into countries outside its native distribution show that this species poses a risk of introductions into other countries where it does not presently occur, particularly through the movement of infested fruit. As with other tephritid fruit fly species, establishment of *B. latifrons* can have significant economic consequences, including damage and loss of food production, as well as requirements for implementation of costly quarantine treatments to permit export of commodities susceptible to infestation by *B. latifrons* and inspection of susceptible imported commodities. Because of the economic importance of *B. latifrons*, reliable methods are needed to detect, monitor, and control this species. We conducted field trials with a wild *B. latifrons* population, supported by the invasive weed, turkeyberry, *Solanum torvum* (Solanaceae), to compare attractiveness of prospective new lures with several attractants that have often been used for detection and/or monitoring of tephritid fruit flies. The tests reported here have again shown higher *B. latifrons* catch in traps baited with alpha-ionol + cade oil relative to traps baited with protein baits. Among the attractants to which both male and female *B. latifrons* are attracted, fly response is significantly better to a Solulys AST–based protein bait than to other attractants tested. Beyond this, there was no significant difference in catch among the (wet) torula yeast baited trap and four (dry) alternative attractants (ammonia, biolure, rainbow plug and cucumber volatile plug). This shows that these dry trap alternatives have a comparable ability to catch *B. latifrons* adults as a wet protein bait trap (though not comparable to a Solulys AST–based wet trap).

Key words: attractants, alpha-ionol + cade oil, Solulys AST, torula yeast, Biolure, ammonium acetate, cucumber volatile plug

Introduction

Bactrocera latifrons is a tephritid fruit fly (Diptera: Tephritidae) which has a host list, based on published field infestation data, of 59 plant species from 14 plant families. The predominant host plant

family is Solanaceae, with published field infestation data for 34 species. The family with the 2nd highest number of documented infested species is Cucurbitaceae, for which there are published field infestation data for 9 plant species (McQuate and Liquido 2013). *Bactrocera latifrons* is of primarily Asian distribution (e.g., Pakistan, India, Sri Lanka, Burma, China [Fujian, Yunnan, Hong Kong, Hainan], Thailand, Laos, Vietnam, W. Malaysia, Singapore, Taiwan, and Brunei) (Carroll et al. 2002), but its range has expanded through introductions into Hawaii (Vargas and Nishida 1985), Okinawa (Japan) (Shimizu et al. 2007), Tanzania (Mwatawala et al. 2007), and Kenya (De Meyer et al. 2013, S. Ekesi, unpublished records). The documented introductions into countries outside its native distribution show that this species poses a risk of introductions into other countries where it does not presently occur, particularly through the movement of infested fruit. As with other tephritid fruit fly species, establishment of *B. latifrons* can have significant economic consequences, including damage and loss of food production, as well as requirements for implementation of costly quarantine treatments to permit export of commodities susceptible to infestation by *B. latifrons* and inspection of susceptible imported commodities.

Because of the economic importance of *B. latifrons*, reliable methods are needed to detect, monitor, and control this species. This species, however, does not respond to either of the strong male lures (methyl eugenol, cue-lure) to which most *Bactrocera* spp. respond (McQuate and Peck 2001). Flath et al. (1994) identified alpha-ionol as a *B. latifrons* male lure. Enhanced attraction was subsequently reported when alpha-ionol was presented with a synergist, cade oil (McQuate and Peck 2001, McQuate et al. 2004). This mixed lure has been shown to be effec-

tive for detection and monitoring of male *B. latifrons* (McQuate et al. 2008). Lab studies have also shown that the introduction of an oxygen atom at the 3-position of the alpha-ionone or alpha-ionol molecules improves the attractiveness of alpha-ionol to male *B. latifrons* (Ishida et al. 2008), with improved attractiveness also found for several compounds derived from these 3-oxygenated derivatives of alpha-ionone and alpha-ionol (Enomoto et al. 2010). No testing, though, has yet been done to see if cade oil would synergize the attractiveness of these compounds. In addition to identification of these male attractants, other identified attractants have included protein baits (McQuate and Peck 2001 [Provesta 621, Integrated Ingredients, Bartlesville, OK, USA], Mwatawala et al. 2007 [specific protein bait used not identified], Mziray et al. 2010 [torula yeast, Scentry Biologicals, Inc., Billings, MT, USA]), and three component lure (Mwatawala et al. 2007: reported attraction based on the capture of a single female *B. latifrons*). One other bait that may have attractant potential for use in detection trapping is the "cucumber essence" bait developed for melon fly, *Bactrocera cucurbitae* (Coquillett) (Siderhurst and Jang 2010). This bait was developed through the identification of cucumber (*Cucumis sativus* L. [Cucurbitaceae]) volatiles that were attractive to melon fly adults and has been shown in field tests to be more attractive to melon fly than a Solulys AST (Roquette America, Inc., Bridgeview, IL) based protein bait. Because cucumber is also a host of *B. latifrons* (Liquido et al. 1994, McQuate and Liquido 2013), it is appropriate to test the attractiveness of this bait to *B. latifrons*.

Establishment of the relative effectiveness of different potential *B. latifrons* adult attractants is of value for detection, monitoring and suppression efforts directed towards *B. latifrons* populations. Here, we present results of field trials with

a wild *B. latifrons* population to compare attractiveness of prospective new lures with several attractants that have often been used for detection and/or monitoring of tephritid fruit flies.

Materials and Methods

Study site. Field trials were all conducted in a cattle pasture in the vicinity of Pepeekeo, Hawaii (UTM Easting, Northing 281611.73, 2195767.77 m Zone 05 Q) in which there were well-developed patches of turkeyberry (*Solanum torvum* Sw.), known to be a good host of *B. latifrons* (Liquido et al. 1994, McQuate and Liquido 2013). This field supported a *B. latifrons* field population of satisfactory size for the present studies and also included *B. dorsalis* (Hendel) and *B. cucurbitae* populations. A total of 8 (Bioassays 1 and 2) or 9 (Bioassay 3) trapping sites were selected, each in the midst of a turkeyberry patch. Trapping sites were all at least 10 m apart. A weather station, maintained in a nearby field, provided temperature, relative humidity and rainfall data throughout the course of all of the field trials.

Fruit collections. In order to provide an assessment of the magnitude of adult fly emergence potential in the test field that could contribute to trap catch, 100 mature green turkeyberry fruits were collected from each trap site (no ground fruits were collected) near the beginning and about the middle of each Bioassay (Bioassay 1: 5/24/2011 & 6/21/2011; Bioassay 2: 8/19/2011 & 9/9/2011; Bioassay 3: 9/20/2011 & 10/18/2011). An additional collection was also made at the end of Bioassay 1 (7/12/2011). Collected fruits were weighed and then placed in screened containers to which sand had been added to serve as a pupation medium. The sand was sieved through a strainer weekly for four successive weeks in order to recover pupating larvae and puparia, which were placed in a small screened cup with sand

and held for adult emergence. Species identification was made of emerged adults as well as unemerged pupae, determination of the latter being made based on the numbers of tubules and form of the prothoracic spiracles (White and Elson-Harris, 1992).

Attractants tested. Baits tested included two protein baits (wet traps) and five dry trap-based attractants (one of which was alpha-ionol + cade oil, the established male lure for *B. latifrons*), together with a water trap (wet trap control) and a dry trap control. Yellow-bottomed Multilure traps (Better World Manufacturing, Fresno, CA) were used for each treatment. Further details on these treatments are presented below:

1) Solulys AST. A 300 ml bait solution was prepared by mixing 8% Solulys AST [Roquette America, Inc., Bridgeview, IL] powder (8.0% w/w) with 4.0% (w/w) borax and 88% (w/w) water. This has been the standard protein bait that we have used for both melon fly and *B. latifrons*.

2) Torula yeast. A 300 ml bait solution was prepared by dissolving 3 torula yeast tablets (ERA International Ltd., Freeport, NY) in 300 ml water. The dried 5.0 grams (plus or minus 0.5 grams) tablet consisted of four parts torula yeast (Lake States Type B) and five parts dry borax decahydrate by weight. This bait has commonly been used for general fruit fly detection trapping in California (California Department of Food & Agriculture 2010).

3) Biolure (or "3-component lure"). For this treatment, three separate chemical release packets (one each of ammonium acetate, trimethylamine and putrescine) were attached to the inside of each trap (Biolure 3-Component Fruit Fly Bait; Suterra, Wenatchee, WA, USA). A yellow sticky card was attached internally to immobilize attracted flies.

4) Rainbow plug. This treatment is an alternative formulation of a "3-component

lure," formulated into a solid plug (Scentry Biologicals, Inc., Billings, MT). It is not sold on the open market but can be used in government (federal and state) trapping programs. A yellow sticky card was attached internally to immobilize attracted flies.

5) "Cucumber Volatile Blend" plug. This treatment incorporated a synthetic lure developed as a plug for attraction of female melon flies (Siderhurst and Jang, 2010) (Scentry Biologicals, Inc., Billings, MT). A yellow sticky card was attached internally to immobilize attracted flies.

6) Ammonia. This treatment included only an ammonium acetate chemical release packet, just one of the three packets used in Treatment no. 3 above (Suterra, Wenatchee, WA, USA). This packet was attached to the inside of each trap. A yellow sticky card was attached internally to immobilize attracted flies.

7) Water. This trap was baited only with water and a surfactant (wet control). Total volume was 300 ml (299 ml water + 1 ml Tween® 20 [Fisher-Scientific, Fair Lawn, NJ]).

8) Yellow sticky card. This treatment included only a yellow sticky card, attached internally to immobilize attracted flies (dry control).

9) Alpha-ionol + Cade Oil. This treatment, the standard male attractant for *B. latifrons*, consisted of 2.0 ml alpha-ionol (4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-ol, obtained from Bedoukian Research, Inc., Danbury, CT) and 1.0 ml rectified cade oil (Penta Manufacturing, West Caldwell, NJ) held on separate 3.8 cm long x 1.0 cm diameter cotton wicks placed in separate plastic baskets suspended at the bottom of the attractant reservoir of the trap. A yellow sticky card was attached internally to immobilize attracted flies.

Bioassays. Three separate bioassays were conducted. The first (*Bioassay* 1) compared catch in traps baited in treatments 1–8 listed above. The second (*Bioassay* 2) compared catch in traps baited with a "Cucumber Volatile Blend" Plug (treatment no. 5 above) in wet traps versus dry traps. The last bioassay (*Bioassay 3*) compared *B. latifrons* catch in traps baited with fresh versus aged alpha-ionol + cade oil versus catch in fresh versus aged deployment of several of the more attractive baits identified in Bioassay no. 1. Further details on these 3 bioassays are presented below.

Bioassay 1. Response to fresh baits (trap catch where bait is aged only up to 7 days) was compared, with fresh bait provided every week. Replication was in time rather than in space. Treatments (one trap each) were randomly assigned to the 8 trapping sites. Traps were serviced twice a week (Tuesday and Friday), with all traps randomly rotated to other sites each week after the Tuesday servicing. At the Friday trap servicing, water was added to liquid baits to replace evaporative water loss over time, returning the volume to 300 ml. In the dry traps, all yellow sticky cards were replaced after the Tuesday trap servicings. Traps were serviced for 8 weeks, through which time each trap was deployed one time at each of the eight trap sites. Initial trap deployment was May 24, 2011, with the final trap service on July 19, 2011.

Bioassay 2. In earlier trapping trials, catch of melon flies in traps baited with the "cucumber volatile blend" was found to be better when deployed as a wet trap rather than as a dry trap (EBJ unpublished data). The wet trap in those trials used a 10% (v/v) polyethylene glycol (PEG) solution (270 ml water; 30 ml PEG) in the trap. The addition of PEG both minimizes water evaporation from the trap and helps preserve any trapped flies. Because of that experience, we compared catch of *B. latifrons* in wet versus dry deployments of traps baited with the cucumber

volatile blend plug. A total of eight traps were randomly assigned to 8 trapping sites (four wet traps and four dry traps, with bait in two traps in each treatment allowed to age over the four week trial and fresh bait provided each week in the other two traps of each treatment). Traps were set out on Tuesday and serviced twice a week (Friday and Tuesday), with all traps randomly rotated to other sites each week after the Tuesday servicing. At the Friday trap servicing, water was added to liquid baits to return the volume to 300 ml. In the dry traps, all yellow sticky cards were replaced on Tuesdays. Traps were serviced for 4 weeks, through which time each trap type was deployed one time at each of the eight trap sites. Initial trap deployment was August 16, 2011, with the final trap service on Sept.13, 2011.

Bioassay 3. In this test, more effective baits identified for *B. latifrons* in Bioassay 1 (ammonium acetate and Biolure) were further tested to compare the response of *B. latifrons* to them relative to the response to the established *B. latifrons* male lure, alpha-ionol + cade oil. Also tested was the effect of lure aging of these three attractants (alpha-ionol + cade oil, Biolure, and ammonium acetate) on response of wild *B. latifrons*. *Bactrocera latifrons* response to traps baited weekly with fresh bait, versus traps in which the lure was weathered up to nine weeks, was compared through weekly servicing. Comparison was also made with response to traps baited with Solulys AST, torula yeast and water, for which fluids were replaced each week. Because of a limited availability of good trap sites at least 10 m distant from other good trap sites, neither the treatments with the fresh baits nor the treatments with the weathered baits were replicated in space. All nine treatments tested (both fresh and aged alpha-ionol + cade oil, Biolure and ammonium acetate, as well as fresh Solulys AST, torula yeast

and water) were initially randomly assigned to trap sites with traps subsequently randomly rotated to other sites each week. Traps were serviced for a total of 9 weeks, through which time each trap type had a one week deployment at each of the nine trap sites. Initial trap deployment was on 20 Sept., 2011, with the last trap service on 22 Nov., 2011.

Statistical analysis. By replicating in time rather than in space (Bioassays 1 and 3), there could be a risk that trapping in a given week could affect (e.g., reduce) trap catch in subsequent weeks, thereby violating the requisite independence of replicate results in parametric statistical analyses. However, the authors feel that the results of the fruit collections show that there was significant continued recruitment to the field population. With that recruitment, combined with natural fly mortality, we feel that trap catch results among weeks can be regarded as being independent, for which parametric statistical analyses are valid.

Bioassay 1. For each fruit fly species, the two catches within a week were added together. Week totals were then square root transformed and difference in catch among treatments tested by ANOVA of square root transformed week totals, with Tukey-Kramer HSD used for means separation. Significance of differences in percentage female response among treatments was tested by ANOVA of arcsine transformed weekly percentage female catch, with Tukey-Kramer HSD used for means separation (SAS Institute Inc. 2010). Untransformed average catches are presented in the figures summarizing average catch results by treatment.

Bioassay 2. The two *B. latifrons* catches within a week were added together. Week totals were then square root transformed and difference in catch among fresh wet and fresh dry treatments was tested by ANOVA of square root transformed week totals.

Bioassay 3. Average weekly *B. latifrons* catches one week after traps were freshly baited (one replicate per treatment each week over 9 weeks) were square root transformed and difference in catch among treatments tested by ANOVA of square root transformed week totals, with Tukey-Kramer HSD used for means separation. Significance of differences in percentage female response among treatments (both among fresh trap catches and among aged trap catches) was tested by ANOVA of arcsine transformed weekly percentage female catch, with Tukey-Kramer HSD used for means separation (SAS Institute Inc. 2010). Change in catch as baits were allowed to age over time was approximated through calculation of a best fit exponential decay curve of trap catch, expressed as the percentage of fresh catch, versus age in weeks, with significance of fit of the decay curve tested by ANOVA. The calculated best fit regression line was then used to estimate the age at which the catch was reduced to 50% of the estimated week one catch.

Results

Fruit collections. *Bactrocera latifrons* and *B. dorsalis*, but no *B. cucurbitae*, were recovered from the turkeyberry collections (Table 1). *Bactrocera latifrons* was recovered from every trap site from every collection date. Recovery averaged 296.8 (± 46.8 [SEM]) adults and unemerged pupae per kg fruit overall, showing that there was a well established *B. latifrons* field population throughout the field trials. Recovery from the three Bioassay 1 collections averaged 157.0 (± 14.8) adults and unemerged pupae per kg fruit, while average recovery for the Bioassay 2 and 3 collections were 400.4 (± 22.6) and 402.8 (± 3.0). The average infestation rates in Bioassays 2 and 3 were higher than any previously reported *B. latifrons* infestation rates in *Solanum torvum* (McQuate and

Liquido 2013).

Bactrocera dorsalis was recovered on 7 of the 8 collection dates, but was found at an average of only 2.7 (± 0.7) (range: 0–5) out of 8 (Bioassays 1 and 2) or 9 (Bioassay 3) trap sites. Recovery averaged 6.6 (± 2.1) adults and unemerged pupae per kg fruit overall. Recovery from the three Bioassay 1 collections averaged 7.2 (±4.6) adults and unemerged pupae per kg fruit, while average recovery for the Bioassay 2 and 3 collections were 10.4 (± 1.0) and 2.0 (± 2.0) adults and unemerged pupae per kg fruit.

Bioassay 1. Over the course of the bioassay, daily temperature and % RH and weekly rainfall averaged 23.2 ℃ (weekly average range: 22.8–23.6 ℃), 83.6 %RH (weekly average range: 81.4–86.3 %RH), and 2.46 cm (weekly average range: 1.3–3.2 cm), respectively. For each fruit fly species, there were significant differences in trap catches among treatments (*B. latifrons* [$F = 8.959$; df = 7,56; $p < 0.0001$], *B. cucurbitae* [$F = 4.292$; df = 7,56; $p = 0.0007$], *B. dorsalis* [$F = 27.473$; df = 7,56; $p < 0.0001$]) (Figure 1). For *B. latifrons*, catch in traps baited with Solulys AST was significantly greater than catch in traps from any other treatment except for traps baited with only ammonium acetate chemical release packets. There were no significant differences in catch among traps baited with torula yeast, Biolure, rainbow plug, cucumber volatile plug, or ammonium acetate, while catch in traps baited with each of these attractants, except for traps baited with the cucumber volatile plug, was significantly greater than catch in either of the control traps. There was no significant difference in percentage female catch among traps baited with Solulys AST, torula yeast, Biolure, rainbow plug, or ammonium packet only ($F = 0.1221$; df = 4,35; $p = 0.9736$), with average percentage female catch ranging from 52.6–59.1%. Average weekly catch

Table 1. Tephritid fruit fly recovery from turkeyberry fruit collections made from turkeyberry patches used for trap sites in Bioassays 1, 2, and 3. Collections in which tephritid fruit flies were recovered are referred to as positive ("pos.") collections. "coll." = collections

| Bioassay number	Date	No. coll. sites	Total no. fruits	Total fruit wt (g)	*B. latifrons*				*B. dorsalis*			
					No. pos. coll.	% pos. coll.	Total no. pupae + adults	No. *B. latifrons* per kg fruit	No. pos. coll.	% pos. coll.	Total no. pupae + adults	No. *B. dorsalis* per kg fruit
1	5/24/2011	8	800	1960.0	8	100	263	134.2	5	62.5	31	15.8
1	6/21/2011	8	800	1824.0	8	100	337	184.8	1	12.5	5	0.003
1	7/12/2011	8	800	1756.6	8	100	267	152.0	2	25.0	10	5.7
2	8/19/2011	8	800	1765.7	8	100	668	377.9	3	37.5	20	11.3
2	9/9/2011	8	800	1695.1	8	100	717	423.0	5	62.5	16	9.4
3	9/20/2011	9	900	1910.2	9	100	775	405.7	0	0.0	0	0.0
3	10/18/2011	9	900	2028.3	9	100	811	399.8	3	33.3	8	3.9
Averages						**100**		**296.8**		**33.3**		**6.6**
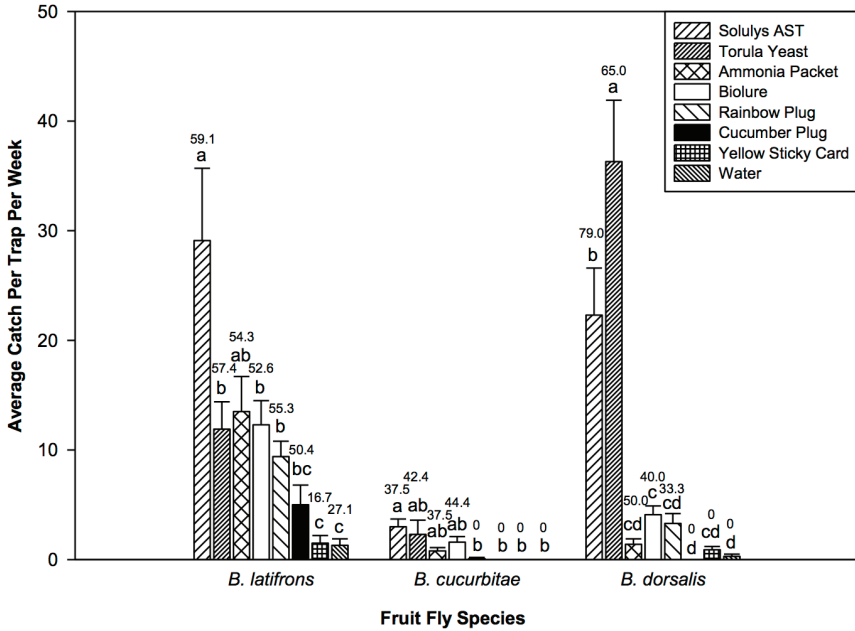
Figure 1. Relative average catch per trap per week (± SEM) of wild *Bactrocera latifrons*, *B. cucurbitae*, and *B. dorsalis* among traps baited with a range of different attractants and in unbaited control traps. Traps serviced twice a week, with trap catches summed for each week's total. Fresh bait provided each week. For each fruit fly species, treatments represented by columns with the same letter at top are not statistically different at the α = 0.05 level. Numbers above the letters indicating statistical significance of differences in average trap catch report the average percentage female catch (Bioassay 1 results).

in traps baited with the cucumber volatile plug, and the two control traps was too low to permit inclusion in the test of significance of differences of percentage female catch among treatments.

For *B. dorsalis*, catch in traps baited with torula yeast was significantly greater than catch in traps baited with Solulys AST, which was significantly greater than catch in traps from any other treatment. Catch in traps from all of the other treatments was quite low by comparison to catch in the Solulys AST and torula yeast treatments. For *B. cucurbitae*, there was no significant difference in catch among traps treated with Solulys AST, torula yeast, ammonium acetate, or Biolure, while catch in each of these treatments

was significantly greater than catch in traps baited with the rainbow plug, the cucumber volatile plug or in either of the control traps. Melon fly catch, overall, was low relative to catch of the other two fruit fly species (see Figure 1).

Bioassay 2. Over the course of the bioassay, daily temperature and % RH and weekly rainfall averaged 23.5°C (weekly average range: 23.2–23.9°C), 84.5% RH (weekly average range: 84.2–85.9% RH), and 4.0 cm (weekly average range: 3.5–5.4 cm), respectively. Throughout this trial, no melon flies or oriental fruit flies were caught, only *B. latifrons*. For *B. latifrons*, catch of flies was low, but significantly greater in fresh dry traps (2.12 ± 0.55 flies/trap/week) compared to fresh wet traps
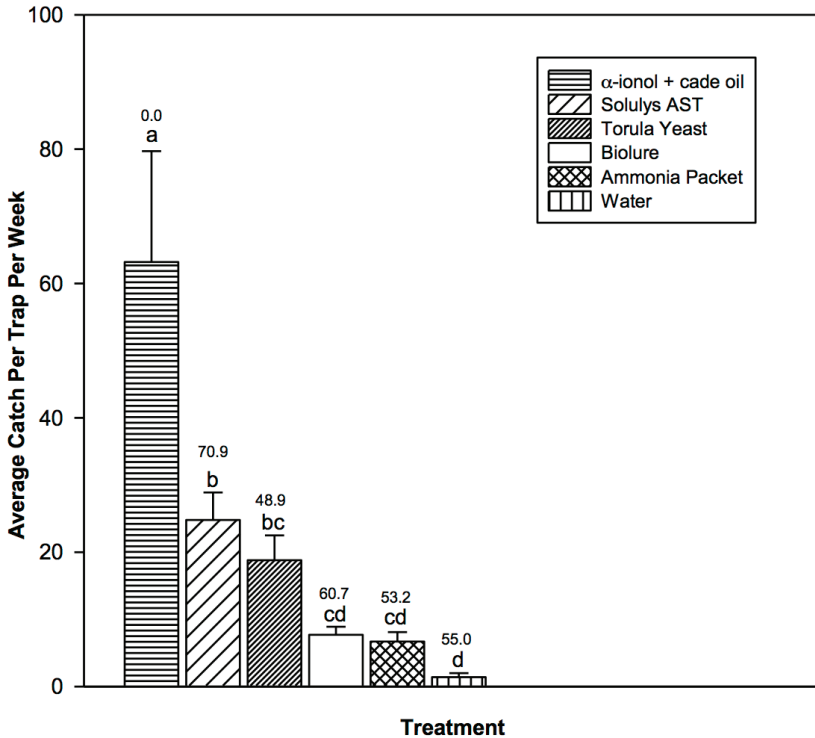
Figure 2. Relative average catch per trap per week (± SEM) of wild *Bactrocera latifrons* among traps baited with a range of different attractants and in unbaited control traps. Traps serviced twice a week, with trap catches summed for each week's total. Fresh bait provided each week. Treatments represented by columns with the same letter at top are not statistically different at the $\alpha = 0.05$ level. Numbers above the letters indicating statistical significance of differences in average trap catch report the average percentage female catch (Bioassay 3 results).

$(0.50 \pm 0.38$ flies/trap/week) $(F = 6.178$; df $= 1,14$; $p = 0.0262$). For both wet and dry traps, the average weekly catch in aged traps was always ≥ the respective average weekly catch in fresh traps. Although the data series was too short to be able to test for significance of trends in catch with increased age of bait, the test did suggest that catch in dry traps was not poorer than catch in wet traps (as had been observed for melon fly [see above: catch was, actually, significantly higher in dry traps]) and that this was true with both fresh and aged baits.

Bioassay 3. Over the course of the bioassay, daily temperature and % RH and weekly rainfall averaged 22.7°C (weekly average range: 22.0–23.6°C), 85.4% RH (weekly average range: 81.8–89.5% RH), and 5.0 cm (weekly average range: 3.1–5.3 cm), respectively. Based on *B. latifrons* catches at freshly baited traps (i.e., catch one week after traps were freshly baited– one replicate per treatment each week over 9 weeks), there was a significant difference in trap catches among treatments $(F = 21.67$; df = 5,48; $p <0.0001$) (Figure 2). Average catch in traps baited with alpha-ionol + cade oil was significantly

greater than catch in traps baited with any of the other treatments tested. The second highest average catch was in traps baited with Solulys AST, with average catch significantly greater than in traps baited with Biolure or ammonium acetate or water only, but not significantly greater than catch in traps baited with torula yeast. Percentage female catch was significantly less in both the fresh and aged alpha-ionol + cade oil treatments (0.0% for each) than in all of the other treatments ($F = 17.46$; df = 7,63; $p < 0.0001$). There was no significant difference in average percentage female catch among the other treatments, which ranged from 44.2% - 70.9% (see Figure 2). The water control trap treatment was not included in the test for significance of difference of percentage female catch among treatments, because of low average weekly catch.

The best-fit exponential decay curve based on reduction of catch at aged traps baited with alpha-ionol + cade oil (as a percentage of the catch at freshly baited traps) over time was significant ($F = 8.98$; df = 1, 7; $p = 0.02$) (see Figure 3). Based on the best-fit equation (% of fresh catch $= 218.15 * e^{-0.286*age\ [weeks]}$), *B. latifrons* catch was reduced to 50% of fresh catch in 5.2 weeks. The best fit exponential decay curves based on reduction of catch at aged traps baited with Biolure or with ammonium acetate alone (as a percentage of the catch at freshly baited traps) over time were not significant (Biolure: $F = 2.93$; df = 1, 7; $p = 0.13$) (ammonium acetate: $F = <0.0$; df = 1, 6; $p = 1.00$), so it was not possible to estimate the weathering time that would reduce catch to 50% of fresh lure catch. However, it was observed that the aged traps for both of these treatments continued to catch flies through the end of the trial, with the catch in the aged traps for weeks 8 and 9 averaging 80% and 104% of fresh trap catches for Biolure and ammonium acetate, respectively.

Discussion

Because there are few local "large" populations of *B. latifrons*, replication in space was not readily possible. Using the alternative of replication in time has the risk that catch variability related to changes in the size of field populations over time might mask treatment-related differences in trap catch. Continued availability of mature green turkeyberry fruits throughout the field trials (see Table 1), however, helped to minimize within treatment trap catch variation, necessary for the demonstration of significant differences in trap catch among treatments.

The tests reported here have again shown higher *B. latifrons* catch in traps baited with alpha-ionol + cade oil relative to traps baited with protein baits. This had previously been reported relative to catch in traps baited with Provesta 621 (10% Provesta 621, 3% borax, and 87% water [w/w]) (McQuate and Peck 2001). Provesta 621 is an autolyzed yeast extract earlier available from Integrated Ingredients (Bartlesville, OK, USA), but is now no longer available. Contrastingly, trap catch in trials in Tanzania were reported to be higher in traps baited with a protein bait (torula yeast, purchased from Scentry, Billings, Montana, USA) relative to traps baited with alpha-ionol + cade oil (Mziray et al. 2010). One difference between the trials in Tanzania and our trials is the method used for "knock-down" of attracted flies: use of a Vapona insecticide strip in Tanzania versus use of a yellow sticky panel in the present studies. It may be that the odor of the Vapona insecticide strip used with the latilure + cade oil traps in Tanzania had a deterrent effect on fly response. Although, *B. latifrons* adults do respond to a torula yeast–based protein bait as used for general fruit fly detection trapping in California, fly response in the present study was found to be significantly higher to a Solulys AST–based protein bait.
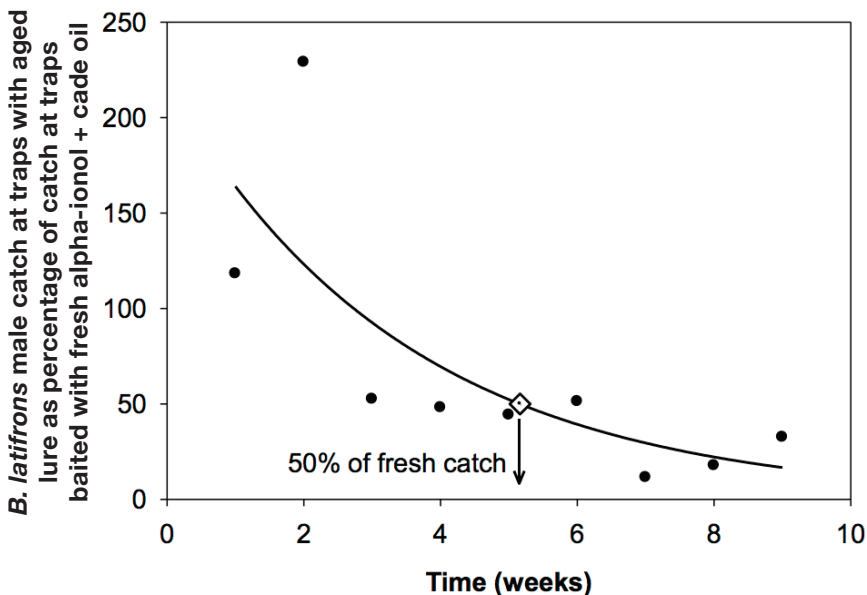
Figure 3. Actual and regressed change in weekly *B. latifrons* male catch at traps with aged lure as percentage of catch at traps baited with fresh alpha-ionol + cade oil. Fresh lure was provided weekly and aged lure was allowed to age over a 9-week period. The estimated age at which trap catch was reduced to 50% of initial fresh catch is presented, as estimated from a best fit exponential decay curve of untransformed trap catch versus weeks of aging (% of fresh *B. latifrons* catch = ae^{-bx}, where a = 218.15, b = 0.286, and x = age in weeks; Bioassay 3 results).

Although the highest *B. latifrons* catch was obtained with traps baited with alpha-ionol + cade oil, only male flies were caught in these traps. Even with males only catch, this lure, with its higher catch, is appropriate for use in trapping programs seeking early detection of invading *B. latifrons*, comparable to the established use of male lures for detection of other tephritid fruit fly species such as Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), melon fly, or oriental fruit fly (California Department of Food and Agriculture 2010). Among attractants tested to which both males and females were attracted, highest catch was achieved with Solulys AST. Among other attractants to which both male and female *B. latifrons* were

attracted, it is interesting to note that there was no significant difference in catch among the (wet) torula yeast baited trap and four (dry) alternative attractants (ammonia, Biolure, rainbow plug and cucumber volatile plug) with that result repeated in both Bioassay 1 and Bioassay 3. This shows that these dry trap alternatives have a comparable ability to catch *B. latifrons* adults as a wet protein bait trap (though not comparable to a Solulys AST–based wet trap). It is also interesting to note, though, that the average catch of *B. dorsalis* in traps baited with these dry alternative attractants was significantly lower than in traps baited with either of the protein baits tested here. The fact that *B. dorsalis* catch in traps baited with torula yeast

was significantly higher than catch in any other traps provides support for the use of a torula yeast bait in *B. dorsalis* detection programs, such as is done in California. It is also good to know that, in cases where Solulys AST is selected for use as a bait in order to optimize *B. latifrons* detection, there is also good response by both *B. dorsalis* and *B. cucurbitae*.

Although there are field data which indicates that cucumber is a host of *B. latifrons* (Liquido et al. 1994, McQuate and Liquido 2013), the reported infestation rate (0.9 *B. latifrons* per kg fruit) is low relative to that for other host plants such as popolo, *Solanum nigrum* [Solanaceae], for which an infestation rate of over 600 *B. latifrons* per kg fruit has been recorded in Hawaii (Vargas and Nishida 1985). Overall, *B. latifrons* field infestation data have been reported for 34 solanaceous plant species, but for only 9 cucurbitaceous plant species (McQuate and Liquido 2013). Among solanaceous plant species, *B. latifrons* field infestation rates of over 400 *B. latifrons* per kg fruit have been reported for 4 different *Solanum* spp. (including popolo) whereas the highest reported infestation rate for a cucurbitaceous plant is 27.9 *B. latifrons*/kg fruit, which was reported for ivy gourd, *Coccinia grandis* (L.) Voigt (Liquido et al. 1994, McQuate and Liquido 2013). Based on these host range details, it seems that volatiles from *Solanum* species hosts may be a better source for development of a host volatile-based attractant than volatiles from a cucurbitaceous host. Preliminary tests using glass McPhail traps in an outdoor olfactometer showed that a slurry of green popolo fruits, adjusted with borax to pH 8.0, produced an average *B. latifrons* catch over 68% as great as catch in a Solulys-AST–baited trap (GTM, unpublished data). Identification and concentration of the primary attractive volatiles could potentially lead to the development of a stronger attractant than the Solulys-AST- based attractant.

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