

FINAL REPORT

WTFRC Project # CH-01-12,

Project title: Factors Affecting Mating By Cherry Fruit Flies
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Objectives:

2001

- Document and describe mating behaviors of the western cherry fruit fly in the laboratory.
- Determine effects of age on mating success and fecundity.
- Document mating behaviors of flies in the field.

2002

- Demonstrate the presence of a mating pheromone in the fly using laboratory wind tunnel and field bioassays.
- Compare the ability of laboratory- and field-collected males to attract females.
- Characterize the chemicals responsible for attraction of females to males.

2003

- Continue to establish presence of pheromones or odors involved in mating behavior.
- Determine visual factors involved in successful mating by flies.
- Determine effect of nutrition on mating success.
- Effects of age on mating and egg hatch success in the laboratory and field.

Significant findings:

2001

- Flies displayed no true courtship behavior; mating was initiated by males that leaped onto females when they were within 1-2 cm.
- Flies that were 3-9 days old mated less frequently than 17-43 d old flies; females exposed to males laid an average of 250 eggs, but mating did not increase egg production.
- Mating was initiated while males were on fruit, and was often completed on leaves; mating occurred frequently whenever temperatures were ≥ 80 °F.

2002

- Extensive laboratory and field bioassays using male flies as a source of odor suggested the females do not respond to chemicals released by males (but further work is needed).
- Neither laboratory nor field-collected males (which may be exposed to different nutrients in the field) attracted females in the absence of visual cues in the laboratory or field.
- Flies mated infrequently during the first 14-19 d after emergence in the field.
- The numbers of eggs in unmated flies were lower than in mated flies, suggesting older flies with higher egg loads are more likely to mate.

2003

- Sex odors did not seem involved in mating success.
- Fruit presence was a visual factor essential for high levels of mating.
- Orange wax cherries attracted more male flies (presumably for mating) than real dark purple fruit, probably because of high substrate (wax) firmness and color.
- Males in the field seemed to prefer orange over green fruit for mating.
- Presence of (dead) males on fruit had no effect on female mating, suggesting the sight of flies on fruit does not stimulate female responses to land on fruit.
- Nutrition in the form of protein did not increase mating, suggesting mating success can be achieved without high protein intake.
- Flies greater than 9 days old showed greatest mating propensity

Methods (2003):

1. Further laboratory studies to determine the presence or absence of a mating pheromone were conducted. Larger tunnel designs and changes in temperatures, light intensity, and wind speed inside tunnels were tested to improve conditions that promote greater or more natural behavior.
2. To provide evidence for visual attraction by females to males, tests were conducted with 20 virgin female flies and 20 males inside screen cages. Sixty cherries were hung from the ceiling of the cages. Fruit were unbaited, baited with dead flies (pinned or glued to fruit), or with live males (that will naturally cling to the fruit). Two-minute observations were made every hour over 8 hours to determine whether females preferential visit fruit with live males. A second test was conducted with artificial red and orange fruit to determine the interaction between color and male presence on mating.
3. Male and female flies were fed diets of sucrose only, sucrose-yeast, and cherry juice only. They were paired in all combinations and exposed to wax domes for egg laying and observed for mating. Eggs were collected, placed on moist filter papers, and hatch rate determined. Egg hatch was used as an indication of successful insemination.
4. Final work was conducted on age-related mating effects in the laboratory and field by pairing males and females of known ages in the lab and by pairing flies collected over the season in the field inside cages.

Results and Discussion:

2001-2003 – Mating Behaviors and Age

Extensive analyses of video recordings indicated that mating behaviors did not involve true courtship. The mating is initiated by males, which wait until females are 1-2 cm away before leaping on them. It appears mate finding is based largely on vision. No evidence of wing fanning or abdominal movement that could be interpreted as dispersing of pheromone was seen in many hours of videotape recordings. A female often resisted the attempts of a male to couple. Once coupled, though, mating lasted up to 73 min (Table 1). The mating pair stayed motionless or walked slowly inside cages. The end of mating occurred when one or the other partner kicked, causing disengagement. No differences were seen in the mating behaviors of 3-9 day old or 17-43 day old flies, although young flies mated less frequently than older flies (Table 1). Mating in the laboratory occurred throughout the day, but mostly during the second half (Fig. 1).

In the field, mating occurred frequently throughout the day in the trees and inside cartons (Fig. 2), whenever temperatures were ≥ 80 °F. Mating in the field was initiated on fruit only, but was often completed on leaves. Males waited on fruit for 2-13 minutes for females to arrive before leaving for other fruit. Males watch as other flies arrive on the fruit or fly by the fruit. In many cases, males did not distinguish between the sexes and attempted to mate with other males. Males often defended the fruit against other males with pawing actions of the forelegs. Mating in the field lasted 18-50 minutes. This long period of coupling may represent a period of increased vulnerability for the flies. Flies were easier to collect when they were coupled than when they were single. In addition, low-dosages of toxic chemicals can perhaps be mechanically transmitted from fly to fly during this process. Such chemicals warrant examination in the future.

Final laboratory work on the effects of age on mating indicated that the average day for first mating is about 10 days (Fig. 3). Age has an effect on mating propensity, but only up to about this age. There was no increase in mating as flies aged.

The laboratory suggested age-related patterns should also be seen in the field. Season-long mating incidence was determined from emergence until flies disappeared in 2002. Mating by flies was determined by observing trees and by pairing males and females that were caught in the field inside cages in Zillah and Roslyn (Fig. 4), WA. In Zillah, mating propensity as determined by observations in trees and inside cups was $< 20\%$ for the first 14 days after first fly detection, when

fruit were small, hard, and green. At Roslyn, mating tendencies were <25% for the first 19 days after flies were seen. These results suggest that during the early part of the season, flies do not mate frequently and thus have a lower chance of bearing fertile eggs than later in the season. If flies are controlled before mating incidence increases, this may reduce the numbers of fertile eggs laid into fruit.

When placed inside pint-size cups in the field, mating usually occurred within 10 min. As supported by earlier laboratory and in-tree observations, no wing fanning or movement of the abdomen by the male, which would suggest the release of a pheromone, was seen. Females also were not agitated or “excited”, which would have indicated they were stimulated by an odor. However, males clearly responded to the sight of females inside cups. Upon seeing a female, a male made jumps to mount her. A pair often struggled momentarily on the bottom of the cage before coupling was complete. In many cases the females resisted the attempts made by the male. However, once coupled, mating was prolonged. Observations were consistently with previous ones.

In Zillah, females that mated had higher numbers of eggs than females that did not throughout most of the season, but especially during the early part. By the end of the season mated and unmated females had about equal numbers of eggs. A similar overall pattern was seen in Roslyn. Flies in Zillah had higher egg loads throughout the season perhaps because these flies had better nutrition, which in turn resulted in greater mating tendencies. This hypothesis was tested in 2003 (below).

2002-2003, Pheromones and Other Factors

Female flies did not respond to male odor in experiments in which males were held inside cages and air was passed over them in experiments conducted in 2002. When the sliding door on the cage holding males was opened, the females did not fly upwind, but rather stayed on the sides of the cages and walked very slowly for hours. The response of female western cherry fruit flies thus were different than that of the European cherry fruit fly, which responded to male odors inside tunnels within 1 hour. A field experiment conducted in an infested backyard cherry tree in June 2002 in which 30 males were held inside paper cups with food and water also yielded the same conclusions. The results suggest female flies are not responsive to odors of males or that the males need to be in a specific physiological or behavioral state to release a pheromone. Because males were kept inside cages or spheres for bioassays and were not hanging on fruit waiting for females (the natural behavior), they may not have been in the right state to release any sex attractants. The background environment may need to be very specific for this to occur. Clearly further work is needed to provide evidence that a pheromone exists, as it does in other fruit flies. If a pheromone is absent, mating may depend mostly if not solely on vision. The question is how flies find one another using vision only; perhaps repeated flights to fruit by females needing to lay eggs are sufficient to achieve successful mating. Further laboratory studies in 2003 to determine the presence or absence of a mating pheromone were conducted. Flies again did not respond strongly to odors despite changes in tunnel designs and temperatures, light intensity, and wind speed inside tunnels.

Several non-chemical factors involved in mating success were tested. One was the presence of fruit. Fruit presence was essential for high levels of mating. When no substrates were present, numbers of matings averaged 1.0; with wax domes on the cage floor, 1.3; with cherries hung from the cage ceiling, 9.3 (4 replicates). Low fruit loads, such as near the end of the season, may cause flies to leave trees in search of mating sites This indicates that sphere traps lure in males that are ready to mate. Another factor was the color of the fruit or fruit-like substrate. Males preferred to rest on wax cherries over real, ripe sweet cherries (Table 2, Experiments 2 and 3). Females tended to visit real cherries more often, perhaps to lay eggs. From this experiment, at least, it appears males and females may not necessarily prefer the same color fruit. If orange sphere traps are more attractive for males, this may help reduce fly populations in the field. Field studies were conducted to determine if males showed evidence for a fruit color preference. Digital pictures were made of 4 trees in Roslyn in July 2003. Images were analyzed and fruit categorized into different colors. Fruit on which males were seen were collected, taken to the laboratory, and placed in the same color categories. Even though

most fruit were green, most males were found on fruit that were yellow/orange or orange/red (Fig. 5), suggesting a preference for riper fruit. This preference may be related to visitation by females. If females also prefer these fruit, then the probability of mating is increased. Female numbers were low, but the available data suggest this was the case.

Although the fruit presence and fruit color are two factors that may affect mating, presence of males on fruit seemed to have no effect on female responses to fruit. The numbers of females that were attracted to wax cherries baited with dead male flies and control cherries were nearly the same (286 versus 279 total female visits for 3 replicates).

Surprisingly, nutrition seemed to have little effect on mating frequency. Male and female flies fed diets of sucrose only, sucrose-yeast, and cherry juice only mated equally (Table 3, Experiments 2 and 3). Total numbers of matings ranged from 0-123 over the 30-day tests. Interestingly, in one sugar-only replicate, there were 123 matings that resulted in a 61.9% egg hatch (n = 113 eggs). However, when this replicate was dropped, the hatch rate of sugar-fed flies was lower than of sucrose-yeast fed flies, partly because the latter ones laid more eggs. The overall hatch rate of this group was 37.4% (total of 463 eggs sampled), compared with 12.4% (total of 114 eggs sampled) in the sugar-only group. Although the numbers of matings in the two groups were equal, but these results suggest insemination of flies with few eggs is lower than when flies produce many eggs.

Overall Conclusions

The results indicate that mating in cherry fruit flies is governed mostly by vision and the flight of females to fruit, where males congregate and where egg laying occurs. There, the males wait for females that visit the fruit for feeding or egg laying. No evidence for a sex pheromone in this process was found. Mating is often completed off the fruit on leaves. It is likely females do not seek the males, but that males detect the females by vision alone. These visual factors may include fruit presence and color. The ingestion of protein with sugar appears to have little effect on mating propensity, but clearly has an impact on egg production. Further research into factors that disrupt the ability of males to mate with females (and vice versa) will aid in managing the fly. The current work indicates that orange sphere traps should be effective in removing large numbers of male flies, especially if fruit loads are low. Trap densities in trees would need to be fairly high to remove large fly numbers. The work also lays a foundation for work on more intensive control methods such as the sterile male technique. Enough is known about the mating behaviors and ecology of the cherry fruit fly to take advantage of advances in this technology. For example, irradiated flies should perform as well as normal flies for this technique to work. Whether this expensive technique will be implemented is question of economics rather than biology.

Table 1. Frequency of mating and mean numbers of matings/day and mean mating duration (minutes) of single male-female pairs of flies different ages in the laboratory.

<u>Age (days)</u>		No.	No. Pairs	% Pairs	No. Matings	Mating
Male	Female	Pairs	Mated	Mating	per day + SE	Duration + SE
3-9	3-9	32	5	15.6	0.08 ± 0.03a	40.12 ± 17.50a
3-9	17-29	25	8	32.0	0.27 ± 0.09a	60.95 ± 13.68a
17-29	3-9	15	4	26.7	0.20 ± 0.12a	73.05 ± 7.48a
17-29	17-29	47	11	23.4	0.44 ± 0.22a	36.76 ± 9.43a
30-43	30-43	16	8	50.0	0.33 ± 0.14a	26.85 ± 11.44a

Means followed by same letters within columns are not significantly different ($P < 0.05$).

Table 2. Effects of nutrition on numbers of mating pairs \pm SE of cherry fruit flies.

<u>Expt 1</u>	<i>N</i>	No.		<u>Expt 2</u>	<i>N</i>	No.	
		<i>N</i>	Matings			<i>N</i>	Matings
Yeast + Sugar + Cherries	5	5.8	± 3.2	Yeast + Sugar + Domes	5	9.2	± 4.7
Sugar Only + Cherries	5	1.6	± 1.4	Sugar Only + Domes	5	26.0	± 24.3

Table 3. Use of orange wax cherries and real dark purple cherries by male and female cherry fruit flies in the laboratory. Twenty of each and 19-35 females and 8-35 males per replicate (*N* = 5).

	Mean total no. of visits + SE over 30 days				
	Diet: Sugar Only		Diet: Sugar-Yeast		
	Wax Cherries	Real Cherries	Females	Wax Cherries	Real Cherries
Females	192 \pm 52	51 \pm 22	Females	275 \pm 126	41 \pm 16
Males	28 \pm 6	1 \pm 1	Males	89 \pm 63	1 \pm 1

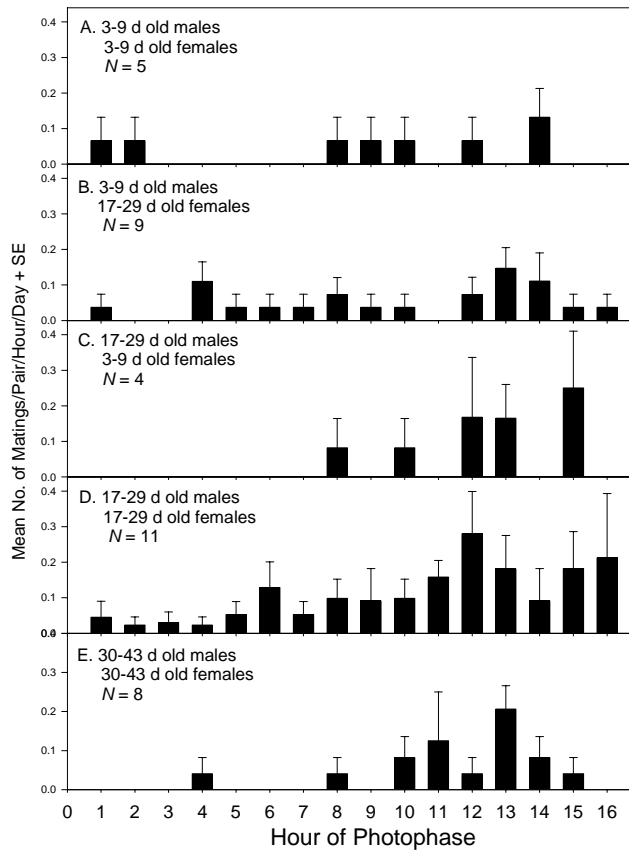


Fig. 1. Time of day effects on mating by cherry fruit flies in the laboratory.

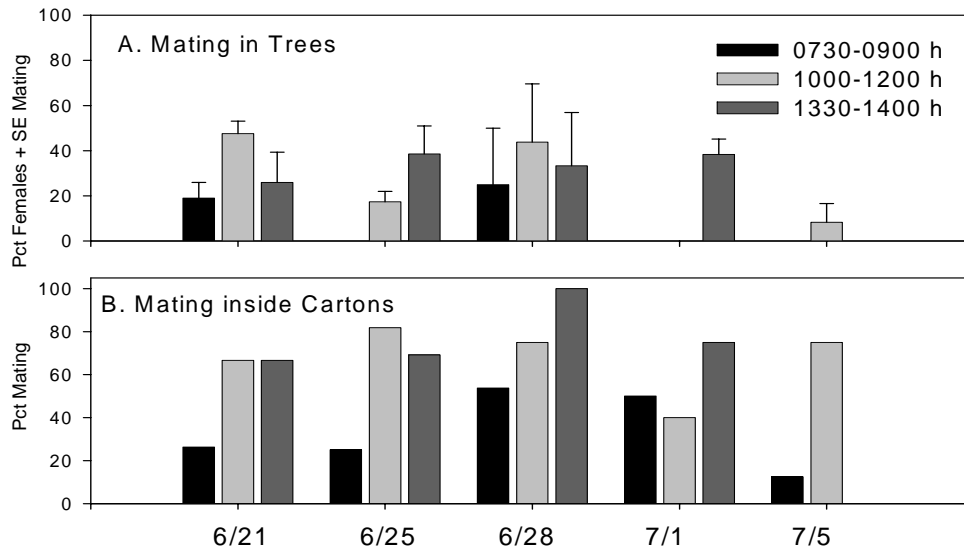


Fig. 2. Field mating incidence by cherry fruit flies in relation to time of day, 2001.

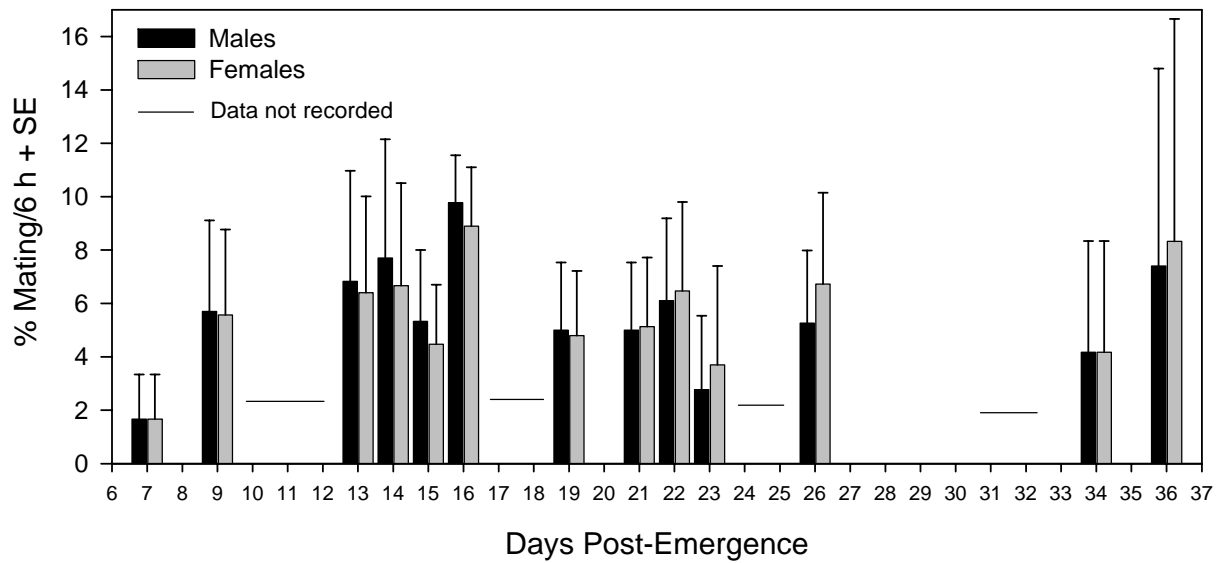


Fig. 3. Mating of cherry fruit flies as a function of age in the laboratory.

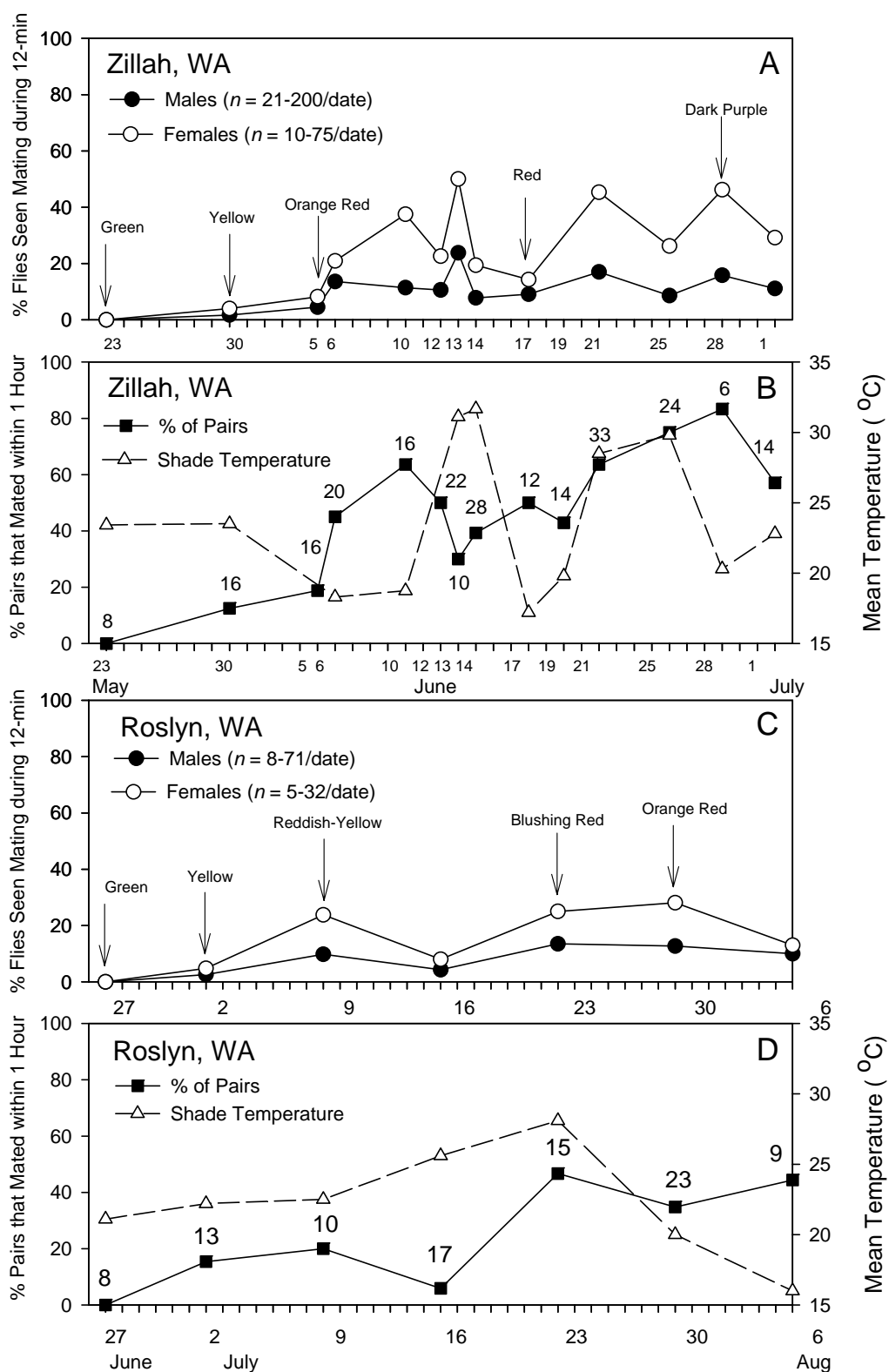


Fig. 4. Percentage of cherry fruit flies seen mating in trees and % that mated inside cups over the season in Zillah and Roslyn, WA, 2002.

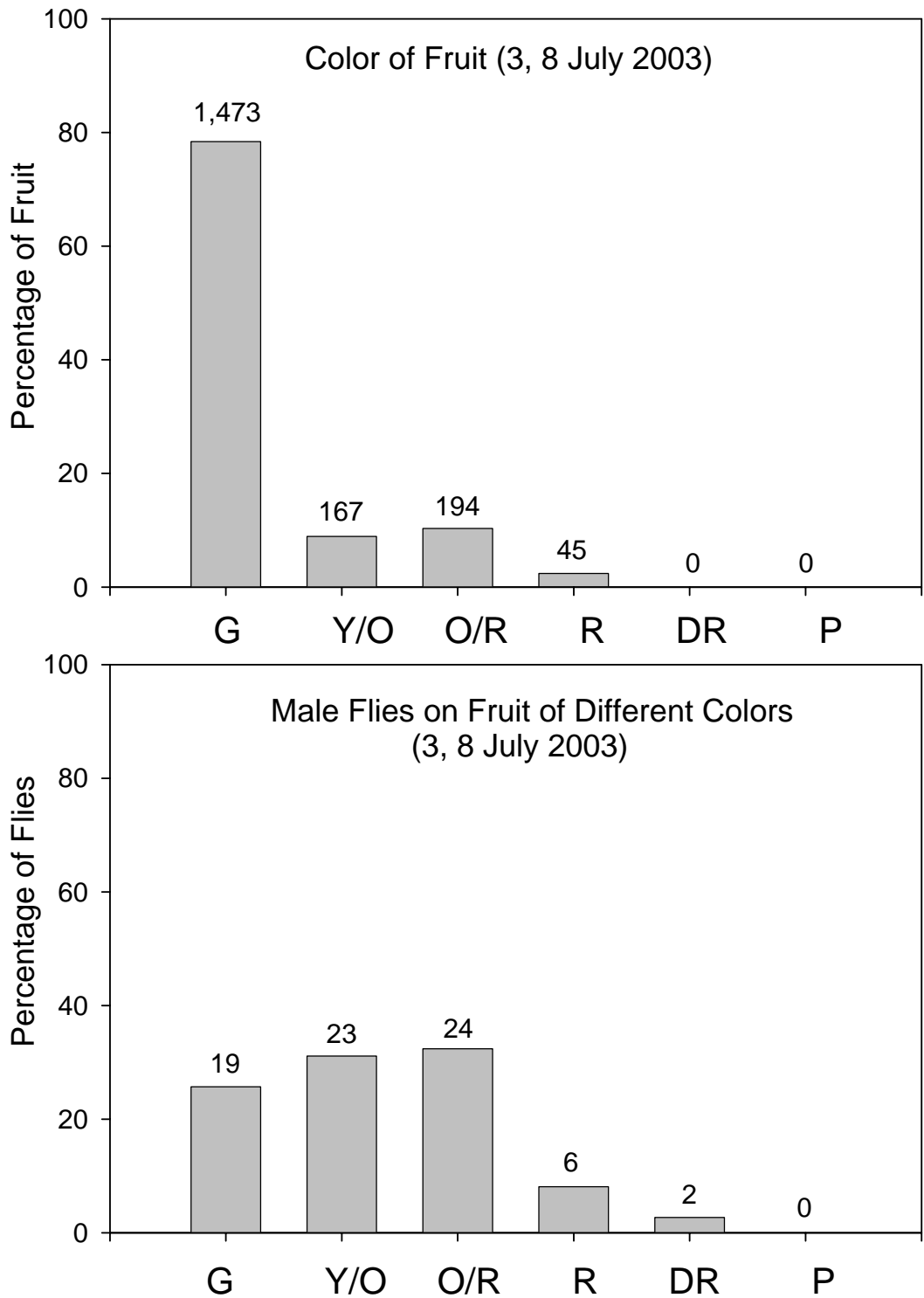


Fig. 5. Color of cherries and numbers of male flies on cherries of different color classes on 4 trees on 3 and 8 July 2003 in Roslyn, WA. G = green; Y/O = yellow/orange; O/R = orange/red; R = red; DR = dark red; P = purple. Numbers above bars are sample sizes

Budget:**Project title:** Factors Affecting Mating By Cherry Fruit Flies**PI:** Wee Yee**Project duration:** 2001-2003**Current year:** 2003**Project total (3 years):** \$51,745.88**Current year requested:** \$10,745.88

Year	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Total	22,000	19,000	10,745.88

Current year breakdown

Item	Year 1(2001)	Year 2 (2002)	Year 3 (2003)
Salaries	15,000	17,500	7,950.80¹
Benefits (%)			795.08
Wages			
Benefits (%)			
Equipment	6,000	1,500	1,500²
Supplies			
Travel	1,000		500³
Miscellaneous			
Total	22,000	19,000	10,745.88

¹One GS-3 or GS-4 (\$9.42 or \$10.58/h), full time, 3 months and 1 GS-3, 50% time, 3 months.

²Cages, cherries; ³To field sites.