EFFECT OF SEX, REPRODUCTIVE MATURITY STAGE AND TRAP PLACEMENT, ON ATTRACTION OF THE BLUEBERRY MAGGOT FLY (DIPTERA: TEPHRITIDAE) TO SPHERE AND PHEROCON AM TRAPS

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ABSTRACT

We compared the performance of 9 cm diameter green spheres and red spheres and Pherocon AM traps, all baited with the same mixture of ammonium acetate and protein hydrolysate, in attracting blueberry maggot flies, *Rhagoletis mendax* Curran, of different reproductive maturity stages and sex. We also evaluated the effect of trap placement in relation to bush canopy (within the top 15 cm of the canopy, or near the base of the bush, 25 cm above ground, within a row) on attraction to these classes of flies. Results of this study showed that captures of flies on red or green spheres were better than on Pherocon AM traps, irrespective of maturity status or sex. Captures of flies were similar among traps placed in the top or the base of the bush, in the case of small bushes (1.2 m high). Traps placed within the top of the canopy captured more flies than those at the base, in the case of larger bushes (1.5-2.0 m high). At both positions, capture patterns were also not dependent on reproductive maturity or sex. Regression analysis between capture ratio of mature females on Pherocon AM/ Spheres and time revealed a significant inverse relationship, which might have been caused by differential aging of these traps. These data show that sphere traps capture more immature flies than Pherocon AM traps, and therefore can be deployed early in the season, when most flies present are immature females. The combination of more effective sphere traps and correct placement strategy depending on bush characteristics can further optimize blueberry maggot monitoring programs.

Key Words: *Rhagoletis mendax*, ovarian development, traps, highbush blueberry

RESUMEN

Comparamos el desempeño de esferas verdes y esferas rojas de 9 cm de diámetro y trampas Pherocon AM, todas cebadas con la misma mezcla de acetato de amonio y proteína "hydrolysate", en atraer moscas de arándano, *Rhagoletis mendaz* Curran, de diferentes etapas de madurez reproductiva y sexo. También evaluamos el efecto de colocación de trampas en relación al dosel del arbusto (dentro de los 15 cm superiores del dosel, o cerca de la base del arbusto, 25 cm sobre el suelo, dentro de la hilera) en atracción a estas clases de moscas. Los resultados de este estudio demostraron que las capturas de moscas en esferas rojas o verdes fueron mejores que en las trampas Pherocon AM, sin respecto del estado de madurez o sexo. Capturas de moscas en esferas rojas o verdes fueron similares entre trampas colocadas en el tope o la base del arbusto, en caso de arbustos pequeños (1.2 m de altura). Trampas colocadas dentro del tope del dosel capturaron mas moscas que aquellas en la base, en casos de arbustos mas grandes (1.5-2.0 m de altura). En ambas posiciones, patrones de captura también fueron independientes de madurez reproductiva o sexo. Análisis de regresión entre promedio de captura de hembras maduras en Pherocon AM /esferas y tiempo revelaron una relación inversa significativa, la cual pudo ser causada por la vejez diferencial de estas trampas. Estos datos demuestran que trampas esféricas capturan mas moscas inmaduras que trampas Pherocon AM, y por lo tanto pueden ser utilizadas temprano en la temporada, cuando la mayoría de las moscas presentes son hembras inmaduras. La combinación de trampas esféricas mas efectivas y una estrategia de colocamiento correcto dependiendo de las características del arbusto pueden optimizar aun más los programas de control y seguimiento de gusanos de arándano.

The blueberry maggot fly, *Rhagoletis mendax* Curran, (Diptera: Tephritidae), is considered the most important pest of commercially grown low and highbush blueberries, *Vaccinium angustifolium* Aiton and *V. corymbosum* L., respectively, in the eastern and midwestern United States and Atlantic provinces of Canada (Prokopy & Coli 1978; Guibord et al. 1985; Vincent & Lareau 1989; Gaul et al. 1995; Teixeira & Polavarapu 2001). Quarantine regulations are in place to prevent blueberry maggot introduction from infested areas in the U.S. and Canada east of the Rocky Mountains. The Blueberry Maggot Certification Program requires growers who want to export fruit from infested areas to non-infested areas in Canada to choose either an IPM-based blueberry maggot management program or a calendar-based spray program (Canadian Food Inspection Agency 1999). Growers involved in an IPM program are required to monitor the presence of adults using baited Pherocon AM traps, deployed at least 2 weeks before the earliest expected emergence. Growers following a calendar spray program are required to start insecticide applications beginning 10 d after the first adult capture in the Pherocon AM traps in the area.

The choice of appropriate trap type is an important factor that can affect the reliability of first detection of adults. The model for most of the research on the roles of visual and olfactory stimuli affecting attraction of *Rhagoletis* flies has been the sibling species apple maggot fly, *R. pomonella* (Walsh). Flies are attracted to yellow panels because of their hue and reflectance, and to dark ~7.5 cm diameter spheres because of their shape and intensity contrast with background light (Prokopy 1968; 1972). Fly visual preferences were attributed to the similarity of the reflectance spectrum of yellow panels with that of foliage, and of the shape and contrast of spheres to those of host fruit (Prokopy 1968). Although the original host of the apple maggot is the hawthorn (*Crataegus* L. spp.), flies show a preference for 7.5 cm diameter spheres, as do other *Rhagoletis* species with smaller host fruits (Prokopy 1977).

Blueberry maggot flies found in the field early in the season are predominantly immature females (Lathrop & Nickels 1932). Therefore, the trap deployed for detecting the onset of adult emergence is required to be very attractive to immature females. Immature females spend more time on foliage, looking for food sources, and mature females on fruit, the site for mating and oviposition (Smith & Prokopy 1981; 1982). To the extent that trap captures reflect fly behavior, it has been suggested that foliage type traps (Pherocon AM) should be more attractive to immature females, and fruit-type traps (spheres) to mature females (Prokopy 1968; Neilson et al. 1984). Recently, Liburd et al. (1998) showed that sphere traps, baited with ammonium acetate and protein hydrolysate, were more attractive to the blueberry maggot than Pherocon AM traps, with an equal amount of bait. However, there are no studies that compare the attraction of baited spheres and Pherocon AM traps to different reproductive maturity stages and sex of blueberry maggot flies.

Captures of *Rhagoletis* flies are greatly increased if traps are placed in locations where the range of visual and odor components includes areas preferred by the insects (Reissig 1975; Drummond et al. 1984; Liburd et al. 2000). Site characteristics, like wind exposure and distance to berries, were found to influence blueberry maggot fly captures in lowbush blueberries (Gaul et al. 1995). In highbush blueberries, Liburd et al. (2000) showed that placing traps 15 cm within the top of the canopy increased captures over the usual placement of 15 cm above the canopy. Immature females, depending on food or fruit abundance, may be present for longer periods or be more active in different locations of the plant canopy than mature females. These factors, together with trap characteristics, should be taken into account when deploying traps to capture immature *R. mendax* females, by placing traps in locations where flies spend more time or are more active.

The objective of this study was to determine the trap type and placement more attractive to immature blueberry maggot females. We evaluated the reproductive maturity of female flies captured on baited Pherocon AM and 9 cm diameter green spheres and red spheres. These traps were placed either 15 cm within the top of the canopy, or near the base of the bush, 25 cm above ground, on bushes of different sizes.

Materials and Methods

Research was conducted during 1999 at 2 highbush blueberry fields, Whitesbog and Chatsworth, located in Burlington County, N.J. The Whitesbog site consisted of a 0.5-ha planting of the cultivar 'Elizabeth'. The Chatsworth site was a 1-ha field planted with the cultivar 'Bluecrop'. Neither field was sprayed or pruned for several years. In Chatsworth, bush height was uniformly about 1.2 m. Bush height in Whitesbog was more variable, ranging from 1.5 to 2.0 m. Bushes in Whitesbog were also larger, with dead wood in the lower portion of the bush.

Traps were hung from metal poles. Two trap placement strategies relative to bush canopy were evaluated. Traps were positioned either within the top 15 cm of the canopy, at a height dependent on bush height, or near the base of the bush, 25 cm above ground (from here on, high and low traps, respectively). In the case of high traps, branches were pruned so that they did not contact the trap. We used Pherocon AM yellow boards (Trécé, Salinas, CA), 9 cm diameter plastic green spheres and red spheres (Great Lakes IPM, Vestaburg, MI). Pherocon AM traps were already baited with ammonium acetate and protein hydrolysate, premixed in the adhesive. Spheres were manually coated with the same average amount of baited adhesive (13 g) as in Pherocon AM traps (Liburd et al. 1998), obtained from the same source. Pherocon AM traps (23 × 28 cm) were hung folded in an ~65° angle, with a V shaped cross-section and the sticky yellow surface facing out.

At both locations, all 3 types of traps were arranged in a randomized complete block design. Main factors were trap type (Pherocon AM, red spheres, green spheres) and placement (high, low). Each of the 6 treatments (3 trap types × 2 placements) was replicated 4 times, yielding 24 traps per experiment. Rows were used for blocks, and traps were 6 m apart. In Whitesbog, blocks were separated by 10 to 30 m to take advantage of more homogenous areas. In Chatsworth, blocks were 8 m apart.

Traps were checked twice weekly, and re-randomized after each inspection. Sampling took place throughout the blueberry maggot fly active season, starting when the first flies appeared in early June, until late July, when the population began to decline. Traps were changed once, 3 weeks after the beginning of the experiment. Captured flies were cleaned with Histoclear (National Diagnostics, Atlanta, GA) and stored in glass vials, in 70% ethanol. Flies were sexed, and females were dissected to determine the degree of ovarian maturation. We categorized females as immature if there was no visible development of ooblasts in the ovarioles. In the apple maggot fly, this was shown to correspond to females of less than 7-8 days of age (Dean 1935; Duan & Prokopy 1994). Previously, females have been classified as mature or immature based on the presence of at least 1 fully developed egg (Neilson et al. 1984; Reynolds & Prokopy 1997). We used a more conservative criterion because we wanted to document differences in trap attraction among recently emerged and older females.

Capture data were square-root transformed ($x + 0.5$) and analyzed by 2-way analysis of variance using PROC ANOVA (SAS Institute 1989) with trap type and placement as main factors. Total trap captures, immature and mature female, and male captures were analyzed separately. Means were separated using Tukey Studentized Range test (SAS Institute 1989). Differences were considered significant at the $P = 0.05$ level.

We calculated the ratios of captures of immature and mature females, on Pherocon AM/Sphere traps (mean captures on Pherocon AM/mean captures on both types of spheres), and on High/Low traps (pooled mean captures on high traps/low traps), for each location. Ratios of captures of immature and mature females at each location were plotted to compare relative trends over time. The relationship between capture ratios within each maturity class and days after first sampling was determined using PROC REG (SAS Institute 1989).

Results

Trap type was a significant factor in captures of all classes of flies in both locations ($P < 0.05$), with the single exception of immature flies in Whitesbog (Table 1). Trap placement was a significant factor for all classes of flies in Whitesbog ($P < 0.05$), but not significant in Chatsworth (Table 1). There was one significant interaction between trap type and placement (Whitesbog) where mature females were captured in relatively lower numbers in low placed Pherocon AM traps than in high placed ones ($P = 0.016$). However, because captures in different trap types and placement follow the same general pattern, we did not perform a separate 1-way analysis of variance. Therefore, for all fly categories we present capture data pooled by either trap type (Table 2) or placement (Table 3). Throughout the sampling period, a total of 17,603 flies were captured in Chatsworth (2,154 immature; 8,765 mature; 6,684 male), and in Whitesbog, 7,169 flies were captured (457 immature; 4,048 mature; 2,664 male).

Green and red spheres attracted 2-3 fold more flies than Pherocon AM traps, in Chatsworth (Table 2), irrespective of sex or female maturity. In Whitesbog, both types of spheres captured more flies in each class, but differences in captures were not significant ($P = 0.077$) for immature females. There were no differences in captures of flies of any class between green and red spheres at either location (Table 2).

With respect to trap placement, in Chatsworth, flies did not show a preference for traps at different positions, irrespective of sex or female maturity (Table 3). In Whitesbog, flies were cap-

Table 1. Results of 2-way ANOVA (F and P- value) for comparisons of captures of adult *R. mendax* (immature and mature females, males, and total captures) on different trap type and placement, from 17 June to 29 July 1999 in Chatsworth and Whitesbog, Burlington County, New Jersey.

Location		Immature		Mature		Male		Total	
Effect	df	F	P	F	P	F	P	F	P
Chatsworth									
Type	2,15	9.85	0.0019	55.59	0.0001	91.39	0.0001	66.00	0.0001
Placement	1,15	1.34	0.26	3.32	0.089	3.86	0.068	0.11	0.74
Type × Placement	2,15	0.95	0.41	1.13	0.35	2.91	0.086	0.73	0.50
Whitesbog									
Type	2,15	3.06	0.077	52.35	0.0001	36.74	0.0001	69.00	0.0001
Placement	1,15	9.68	0.0071	61.48	0.0001	147.39	0.0001	146.36	0.0001
Type × Placement	2,15	1.53	0.25	6.10	0.016	0.44	0.65	1.65	0.23

TABLE 2. EFFECT OF TRAP TYPE ON CAPTURES OF ADULT *R. MENDAX* (IMMATURE AND MATURE FEMALES, MALES, AND TOTAL CAPTURES) FROM 17 JUNE TO 29 JULY 1999 IN CHATSWORTH AND WHITESBOG, BURLINGTON COUNTY, NEW JERSEY.

Location	Mean ± SEM no. flies per trap			
Type	Immature	Mature	Male	Total
Chatsworth				
Red Sphere	111.6 ± 8.6 a	453.3 ± 47.3 a	395.0 ± 59.5 a	959.4 ± 119.6 a
Green Sphere	100.4 ± 14.9 a	448.0 ± 45.9 a	330.0 ± 33.4 a	878.9 ± 88.9 a
Pherocon AM	57.3 ± 23.3 b	194.4 ± 19.0 b	110.5 ± 10.8 b	362.1 ± 32.0 b
Whitesbog				
Red Sphere	19.6 ± 3.9 a	206.5 ± 20.1 a	136.5 ± 29.5 a	362.6 ± 49.9 a
Green Sphere	21.5 ± 4.8 a	203.3 ± 25.0 a	138.0 ± 32.5 a	362.8 ± 59.5 a
Pherocon AM	16.0 ± 5.1 a	96.3 ± 22.4 b	58.5 ± 15.2 b	170.8 ± 39.8 b

Means in the same column followed by the same letter (at each location) are not significantly different ($P = 0.05$, Tukey Studentized Range Test).

tured in larger numbers in traps placed in the top of the canopy (Table 3). At this location, high traps captured 44% more immature females, 2-fold more mature females, and 3-fold more males.

The ratios of captures of immature females between Pherocon AM/Spheres and High/Low traps generally follow a similar trend to those of mature flies, in the sampling dates where both classes were captured (Fig. 1). Ratios of captures in Whitesbog are much more variable than in Chatsworth, possibly reflecting increased heterogeneity in bush size. However, ratios of captures of immature and mature females still follow a similar pattern. Regression analysis revealed that, in Chatsworth, for mature flies, there was a significant inverse relationship (Table 4) between Pherocon AM/Sphere trap capture ratio and time ($P = 0.031$). There was also a significant inverse relationship between the High/Low trap capture ratio and time in the case of immature females in Whitesbog ($P = 0.047$). No significant relationships ($P < 0.05$) were found in Whitesbog.

DISCUSSION

Results of this study show that baited red or green spheres were better than Pherocon AM traps in attracting blueberry maggot adults, irrespective of sex or female maturity. These results support the use of baited sphere traps early in the season, when most flies found in the field are immature females. Trap placement in relation to bush canopy was also found to influence fly captures. In case of small bushes (1.2 m), traps placed 15 cm within the top of the canopy were as attractive as those placed near the base of the bush, 25 cm above ground. Where bushes are larger (1.5 to 2.0 m), with fruit and leaves mostly on the top of the canopy, flies were captured in larger numbers on high traps. Liburd et al. (2000) have shown that total captures of flies on traps placed within the top 15 cm of the canopy were equal to or better than traps placed 45 cm above ground for bushes of size comparable to the large ones in this study (1.5 to 1.8 m). These data provide information on

TABLE 3. EFFECT OF TRAP HEIGHT ON CAPTURES OF ADULT *R. MENDAX* (IMMATURE AND MATURE FEMALES, MALES, AND TOTAL CAPTURES) FROM 17 JUNE TO 29 JULY 1999 IN CHATSWORTH AND WHITESBOG, BURLINGTON COUNTY, NEW JERSEY.

Location	Mean ± SEM no. flies per trap			
Height	Immature	Mature	Male	Total
Chatsworth				
High	81.3 ± 9.1 a	341.0 ± 41.5 a	301.3 ± 53.6 a	723.5 ± 100.5 a
Low	98.3 ± 19.0 a	389.4 ± 53.2 a	255.8 ± 41.9 a	743.4 ± 111.6 a
Whitesbog				
High	22.5 ± 3.9 a	208.2 ± 17.1 a	166.3 ± 22.9 a	396.9 ± 42.3 a
Low	15.6 ± 3.3 b	129.2 ± 23.4 b	55.8 ± 9.4 b	200.5 ± 34.3 b

Means in the same column followed by the same letter (at each location) are not significantly different ($P = 0.05$, Tukey Studentized Range Test).

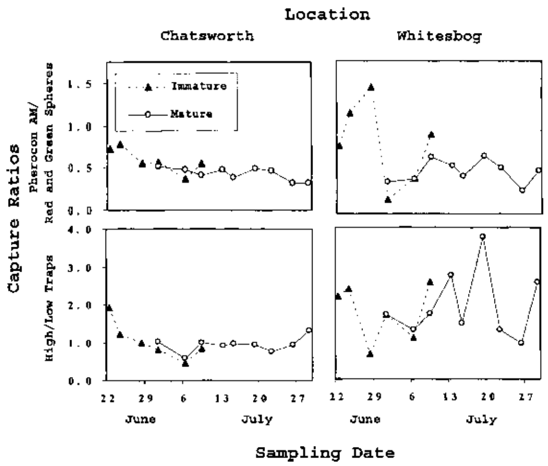

Fig. 1. Ratios of captures of immature and mature blueberry maggot fly females between Pherocon AM/ Sphere traps, and High/Low traps, throughout the sampling period.

the best placement of traps with respect to bush physical characteristics, for optimizing captures of immature flies early in the season.

Contrary to previous expectations (Prokopy 1968; Neilson et al. 1984), baited spheres captured significantly more immature females than Pherocon AM traps. The different behavioral preferences exhibited by the blueberry maggot fly for foliage and fruit (Smith & Prokopy 1981; 1982), may not translate into higher trap captures of immature females on baited Pherocon AM traps than on sphere traps. Our study suggests that immature females, like mature females, are receptive to the ammonia stimulus emanating from the bait, and the shape and intensity contrast stimulus from the spheres. There are few studies focusing on relative trap attraction to *Rhagoletis* flies regarding sex and reproductive maturity. Prokopy (1968) evaluated the maturity of female apple maggot flies captured on unbaited yellow boards and red spheres. The percentage of immature females was larger in yellow boards, but the absolute numbers captured were very similar. Neilson et al. (1984) captured slightly more blueberry maggot immature females on baited Pherocon AM traps than on 8.5 cm diameter unbaited red spheres. This is the first study that compares the attraction of baited sphere and Pherocon AM traps to different reproductive maturity stages and sex of blueberry maggot flies.

In this study we found no differences between the performance of red and green spheres for each fly category. Liburd et al. (1998) also had found little difference between red, green, yellow, and blue spheres. These results seem to indicate that the blueberry maggot fly has a response to colored spheres similar to the apple maggot fly, using shape and intensity contrast to detect traps (Owens & Prokopy 1986). Differences in background in relation to red and green spheres were shown to have a complex influence on apple maggot fly captures (Prokopy 1986). Here, differences in background (sky and foliage for traps placed in the canopy, foliage and ground for those near the base of the bush) do not seem to be the major influence on trap captures.

Physical characteristics of the bush were an important factor in captures of all classes of flies. In the case of large bushes, the results are clearly in favor of placement of traps 15 cm within the top of the canopy. Low traps placed near tall bushes were close to dead or non-bearing branches. These areas are probably not very attractive for flies looking for either food sources or fruit for oviposition. Shade from large bushes might have also contributed to the poor performance of low traps. Low Pherocon AM performed poorly when compared to high Pherocon AM traps, possibly because they require flies to go lower to come into visual contact with the yellow surface (facing down), in contrast to spheres, which are visible from all angles. Even though traps placed 25 cm

TABLE 4. REGRESSION ANALYSIS (PARAMETERS, R^2 AND P VALUE) BETWEEN CAPTURE RATIOS OF ADULT *R. MENDAX* (IMMATURE AND MATURE FEMALES) ON PHEROCON AM/SPHERE TRAPS AND HIGH/LOW TRAPS, AND DAYS AFTER FIRST SAMPLING, IN CHATSWORTH AND WHITESBOG, BURLINGTON COUNTY, NEW JERSEY. RATIOS INCLUDE CAPTURES OF IMMATURE FEMALES FROM 17 JUNE TO 9 JULY 1999, AND MATURE FEMALES FROM 1 JULY TO 29 JULY 1999.

Location	Immature				Mature			
Ratio	a	b	R^2	P	a	b	R^2	P
Chatsworth								
Pherocon AM/Sphere	0.75	-0.017	0.60	0.07	0.57	-0.006	0.51	0.03
High/Low	1.59	-0.061	0.67	0.05	0.73	0.008	0.15	0.30
Whitesbog								
Pherocon AM/Sphere	1.02	-0.024	0.11	0.52	0.47	0.000	0.00	0.98
High/Low	1.82	-0.008	0.00	0.90	1.61	0.013	0.02	0.73

above the ground were somewhat hidden, a large number of flies were still captured in these traps, probably because of a strong effect of the ammonia bait. Aluja & Prokopy (1993) found that if the visual stimulus is strong, odor did not increase the probability of flies finding a fruit, but flies do increasingly rely on odor to find fruit when the visual stimulus becomes weaker.

In smaller bushes (1.2 m), capture patterns were very similar at both 15 cm within the top of the canopy, or 25 cm above ground. Flies of all categories were captured in similar numbers at either trap placement. Gaul et al. (1995) found that placing traps close to fruit increased captures in lowbush blueberries. Behavioral observations have shown that both male and mature females tend to visit and spend more time on fruit, the site for mating and oviposition (Smith & Prokopy 1981; 1982). Lack of preference for high or low traps seems to indicate that if fruit and foliage is more evenly distributed, as on smaller bushes, flies do not restrict their search for food sources or mating and oviposition sites to any particular area of the canopy. Sheltered Pherocon AM traps captured more flies than those exposed to wind (Gaul et al. 1995). This suggests that factors like wind speed at different heights of the canopy, light intensity, temperature, and humidity might also control fly movement and influence captures on low traps.

The significant inverse relationship between captures of mature females on Pherocon AM/Sphere traps and time might be caused by several factors. First, we classified flies as mature if there was any visible developing ooblasts in the ovarioles. It is possible that flies might experience a shift in preference towards spheres after reaching the maturity threshold we established in this study. Given the synchronized pattern of emergence and maturity of the population in the field, the slight trend we observed is not what would be expected if preference were dependent on fly maturity. Second, Pherocon AM and sphere traps may have different ammonia release rates, because of differences in surface area causing slower bait depletion on spheres compared to Pherocon AM traps. In a previous study, Liburd et al. (2000) showed that Pherocon AM and sphere traps experienced a drop in captures at 11 and 40 d, respectively. Finally, attraction to Pherocon AM traps is strongly dependent on yellow hue and reflectance (Prokopy 1972). It is possible that decreased transparence of the adhesive, accumulation of detritus, or pigment decay may affect captures. Spheres, on the other hand, are attractive because of shape and light intensity contrast, which does not change with time. There was also a significant inverse relationship between captures of immature females on High/Low with time, in Chatsworth. Given the few data points we could use in the regression, the reasons for the week relationship (P = 0.047) are not clear. In Whitesbog, variability on trap captures between sampling dates, caused by increased heterogeneity in bush size, might have obscured any patterns.

Results of this study indicate that the use of red or green 9 cm diameter plastic sphere traps, baited with ammonium acetate and protein hydrolysate, can significantly increase captures of adult blueberry maggot flies, including immature females, over Pherocon AM traps. In the case of small bushes, captures of flies on traps placed 15 cm within the top of the canopy are equivalent to captures on traps placed 25 cm above ground, for all categories of flies. Smaller bushes are the norm in commercial blueberry plantings. One advantage of low placement is that it does not require pruning of the bush. In case of larger bushes, placement within the top of the canopy is better than near the base of the bush. Blueberry maggot certification programs, either IPM- or calendar-based, that require monitoring of adult presence can benefit from the increased accuracy of spray recommendations as a result of using more effective trap type and placement appropriate to given bush size.

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