MOSQUITO AREA REPELLENTS: LABORATORY TESTING OF CANDIDATE MATERIALS AGAINST *AEDES AEGYPTI*[1]

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ABSTRACT Selected petroleum oil fractions, essential oils, pyrethrum, pyrethroids and commercial repellents were tested for area repellency in the laboratory against *Aedes aegypti*. A petroleum fraction with a high naphthalene content, citronellal, geraniol, allethrin, esbiol, D-*trans*-allethrin, N,N-diethyl-*m*-toluamide and Mosquito Beater[R] exhibited the best area repellency of the materials tested and warrant continued laboratory testing. Mosquito Beater[R], a commercially available area repellent with methylated naphthalenes, naphthalene, and petroleum distillate comprising approximately 30% of the active ingredients was the most effective material tested.

The use of repellents is a practical means of reducing the biting activity of bloodsucking arthropods and for the interruption of arthropod-borne disease transmission. An effective area repellent, which would substantially reduce or eliminate arthropod biting activity in the treated area, would fill the gap which currently exists between conventional control with insecticides and topical repellents. Several groups of compounds, including petroleum fractions, essential oils, pyrethrums and pyrethroids, have been attributed with area repellency against biting dipterans.

Several authors (Bunker and Hirschfelder 1925, Ginsburg 1935, Hocking and Lindsay 1958, Horsfall 1959, and Lopp and Buchanan 1959) have reported that petroleum fractions exhibit mosquito repellency. Velsicol AR50, a solvent containing methylated naphthalenes, and diesel fuel oil were repellent against several species of Diptera, including female *Culex tarsalis*, in olfactometer tests (Hocking and Lindsay 1958). Horsfall (1959) reported that "prehatch larvicide" treatment with DDT in a petroleum fraction on vermiculite granules prevented annoyance from *Aedes* and *Psorophora* mosquitoes for up to 4 days after treatment. Lopp and Buchanan (1959) applied the same petroleum fraction, with and without DDT, and reported a 90–100% reduction in landing counts 4 days after treatment as compared with landing counts before treatment. They concluded that the solvent alone was responsible for the area repellency. Mosquito Beater[R] is a commercially available area repellent with polymethylated naphthalene (16%), naphthalene (4.5%), and a petroleum distillate (9.0%) as the primary active ingredients. This material was highly effective for up to 24 hr after application in lowering landing rates of *Aedes* and *Anopheles* mosquitoes and black flies in the area treated (Means 1973, 1978).

Essential oils have long been used to repel mosquitoes (Berry et al. 1965, Bunker and Hirschfelder 1925, McCulloch and Waterhouse 1947). Christophers (1947) and Sarkaria and Brown (1951) reported that citronellal (3,7-dimethyl-6-octen-1-ol-1, oil of citronella) exhibited the strongest repellency of the compounds tested against *Aedes aegypti*, while Bunker and Hirschfelder (1925) found citronellol (3,7-dimethyl-6-octen-1-ol, oil of citronella) was the most effective repellent of 57 compounds tested in the

[1] The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense. Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the Department of the Army or the Department of Defense.

field. Many essential oils belong to the class of compounds known as terpenes. Berry et al. (1965) and Langford et al. (1966) examined a commercial material (Desdall, manufactured by Sinder Corp., New York, NY) consisting principally of terpenes, terpene alcohols, and terpene oxide for area repellency. They found this material slightly repellent. Other essential oils with reported repellent activity include: geraniol (oil of citronella) and cineole (oil of eucalyptus) (Bunker and Hirschfelder 1925), methyl eugenol (Huon pine oil) (McCulloch and Waterhouse 1947), and thujic acid (oil of cedar wood) (Hach and McDonald 1971).

Mosquito coils produce a pyrethrum-containing smoke on burning, which exhibits repellency, knockdown, and kill against mosquitoes and other flying insects (Maciver 1963). Glynne Jones and Sylvester (1966) reported that 0.1% pyrethrum sprayed in huts deterred 90% of *Anopheles minimus* from entering the night following spraying and persisted in diminishing effect for 4 days. The synthetic pyrethroids currently available have much higher insecticidal activity and are more stable to environmental conditions than pyrethrins. Repellent activity has been observed with some pyrethroids, and selected compounds were tested for area repellency. Standard repellents, used for topical and cloth application, also have been tested for area repellency (Gorham 1974).

In this study, selected petroleum oil fractions, essential oils, pyrethrum, pyrethroids and commercially available repellents were tested in the laboratory for area repellent activity (Table 1).

MATERIALS AND METHODS

TEST MATERIALS. Sources of the materials tested were as follows: cedrene, cineole, citronellol and geraniol from Pfaltz and Bauer, Inc., Stamford, CT; citronellal (95%) from Sigma Chemical Co., St. Louis, MO; deet (N,N-diethyl-*m*-toluamide) (deet plus isomers 75% in ethanol) from Airosol Co., Inc., Neodesha, KS; 2-ethyl-1,3-hexanediol and dimethyl phthalate from Niagara Chemical Division, Middleport, NY; dibutyl-phthalate from Union Carbide, Jacksonville, FL; Indalone (butyl-3,4-dihydro-2,2-dimethyl-4-oxo-2,4-pyran-6-carboxylate) from K&K Laboratories, Plainview, NY; petroleum fractions (boiling point in the kerosene and fuel oil range) from Chevron Research Co., Richmond, CA; pyrethrum concentrate (22.1% pyrethrum I and 18.3% pyrethrum II) and pyrethroids from McLaughlin, Gormley King Co., Minneapolis, MN; and Mosquito Beater^{R} (EPA Reg. No. 4-123, Active ingredients: methylated naphthalenes 16.0%, napthalene 4.5%, beta-butoxy beta-thiocyano diethyl ether 1%, butoxypolypropylene glycol 0.5%, petroleum distillate 9.0%; inert ingredients: 30–50 mesh exfoliated vermiculite granules 69%) from Bonide Chemical Co., Inc., Yorkville, NY.

MOSQUITOES TESTED. All tests were conducted with *Aedes aegypti* (L.). Mosquitoes were reared and maintained at 27° ± 5° C and 80% ± 10% relative humidity under a 12:12-hr photoperiod incorporating 1-hr of simulated sunrise and 1-hr of simulated sunset. Daytime illumination was held at 320 lx. Larvae were reared on a diet of Purina Guinea Pig Chow (ground to 40-mesh), brewer's yeast and undefatted desiccated powdered liver (ratio by weight, 4:4:1). Adult mosquitoes were maintained on 10% sucrose ad lib. Testing was conducted on nulliparous mosquitoes 5–15 days of age.

Test cage: Area repellent laboratory tests were conducted in a 60×60×60 cm test cage (Fig. 1), the sides of which were constructed of 0.32 cm (1/8") Plexiglas^{R} with the top and bottom areas covered with 16-mesh nylon screen. The front panel contained a 8×21 cm sliding door in the lower right corner and a 20×30 cm opening in the upper left corner was closed with a 60 cm length of 20 cm (8") diameter tubular stockinette. Glass plates supporting the materials to be tested were placed into and removed from the test cage through the sliding door, and the

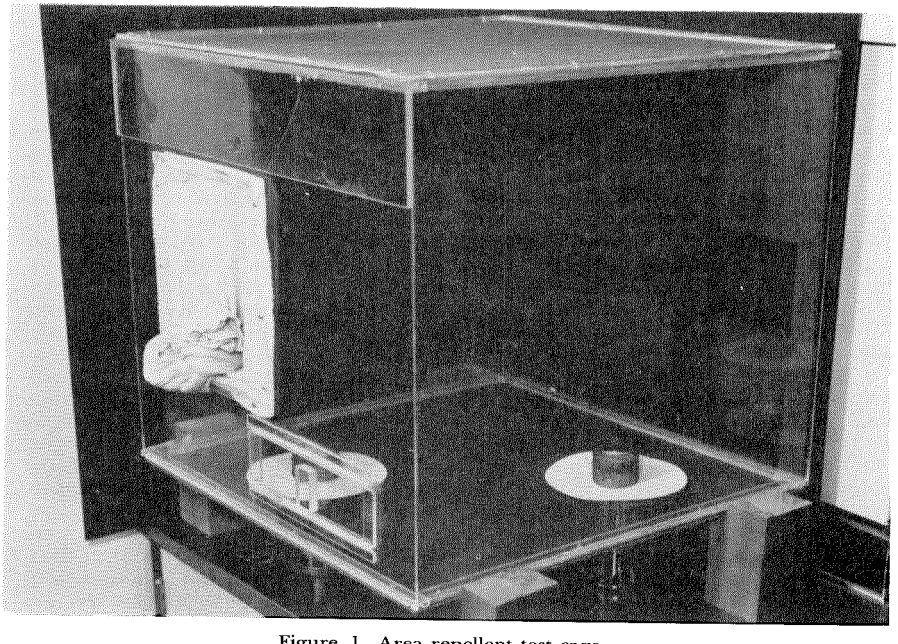

Figure 1. Area repellent test cage.

mosquitoes, test animals and animal cages were placed into and removed from the test cage through the stockinette. The test cage was supported 15 cm above the work table on 4 wooden blocks to facilitate air movement through the cage. Two inverted 1000 cm beakers were used under the test cage to support the glass plates and to prevent the nylon mesh floor from sagging.

Cages for the test animals (suckling mice) were constructed from 8-mesh brass screen (Fig. 2). These cages consisted of a 6 cm high screen cylinder 6 cm in diameter. A circular section of screen was fastened 3 cm from the end of the cylinder to support the suckling mice above the test material.

AREA REPELLENT TEST PROCEDURES. Area repellent laboratory tests were begun by transferring with an aspirator 50 adult female *Ae. aegypti* mosquitoes to the test cage. The mosquitoes were allowed to acclimate for 10–15 min while the test materials were prepared. Test compounds were diluted or dissolved in 3.0 ml acetone and spread evenly on an 18.5 cm diameter sheet of Whatman No. 1 filter paper centered on a 20×20×0.32 cm glass plate. (Thin layer chromatography plates were used.) A control paper received 3.0 ml of acetone. Papers were air dried for 5 min to allow the solvent to evaporate and then transferred, on the glass plate, to the test cage containing the mosquitoes. The glass plates were evenly spaced along the diagonal of the test cage floor. The location of the control and experimental plates were alternated after each test. Hexane was used as the solvent and control for tests run on pyrethrum. Mosquito Beater^{R} was tested by evenly

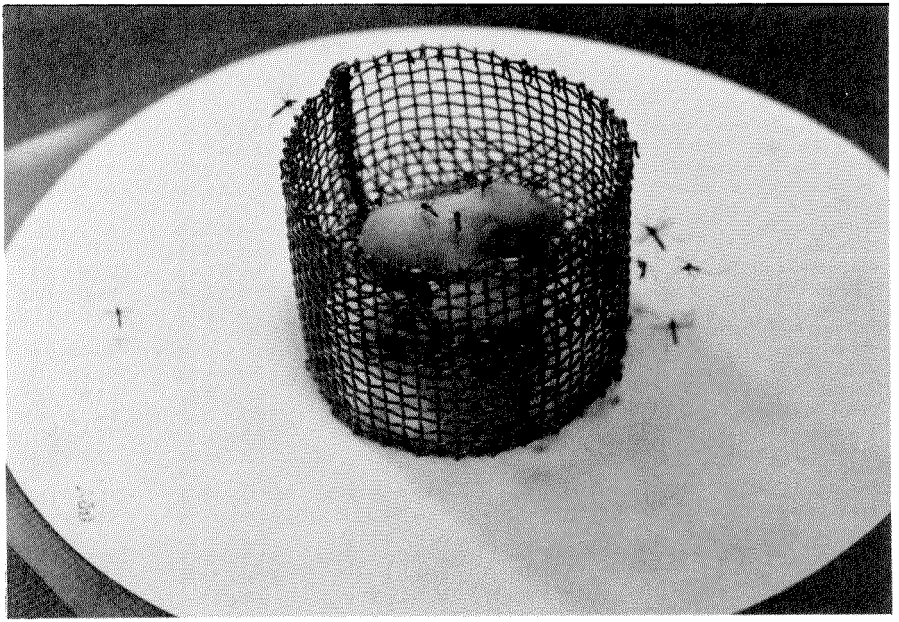

Figure 2. Mosquitoes feeding on suckling mice held above a control treated filter paper.

dispersing 0.32 gm material (0.1 gm active ingredients) in a glass petri dish (14.6 cm diameter) with the control dish containing 0.22 gm of untreated vermiculite granules.

Area repellent tests were initiated by centering a cage containing 2 suckling white mice (4–6 days of age) on each of the filter papers. Biting counts were than recorded at 2 min. intervals for 20 min. Tests with biting count totals on the control mice of less than 50 per 20 min were rejected and the test repeated. At the end of the testing period mosquitoes were transferred to a small holding cage and given 10% sucrose ad lib. A mortality count was taken 24-hr after testing.

Percent protection for each test was calculated as follows:

$$\text{Percent Protection} = 100 \times \frac{(\text{Control—Exp. Biting Count})}{(\text{Control Biting Count})}$$

Five tests per material were conducted on petroleum oil fractions and essential oils. Three tests per material were conducted on pyrethrum, pyrethroids, and the commercially available repellents.

RESULTS AND DISCUSSION

The mean biting counts and mean percent protection for the materials tested are given in Table 1. Efficacy of these materials as area repellents is probably due to a combination of the repellency of the compound and its volatility. Materials

Table 1. Laboratory area repellent test results against *Aedes aegypti* (L.)

Test Material	Amount Tested	Biting Counts[a]		Percent[b] Protection
		Control	Experimental	
Petroleum Oil Fractions				
High napthalene content	1.0 ml	105.4 ± 26.8	10.2 ± 9.8	90.9 ± 7.9
High paraffin content	1.0 ml	106.0 ± 29.3	23.4 ± 6.7	77.7 ± 3.6
High alkylbenzene content	1.0 ml	77.4 ± 25.1	52.0 ± 23.8	33.7 ± 22.3
High naphthene content	1.0 ml	80.6 ± 19.9	31.0 ± 22.0	61.7 ± 23.1
Essential Oils				
Cedrene	0.1 ml	186.0 ± 47.1	66.4 ± 38.4	63.7 ± 26.9
Cineole	0.1 ml	162.0 ± 56.1	65.4 ± 56.4	59.3 ± 35.2
Citronellal (95%)	0.1 ml	195.4 ± 28.6	8.2 ± 10.5	95.9 ± 5.1
Citronellol	0.1 ml	186.0 ± 61.6	28.6 ± 18.5	83.8 ± 10.3
Geraniol	0.1 ml	189.0 ± 23.5	16.8 ± 16.2	90.2 ± 11.0
Pyrethrum and Pyrethroids				
Pyrethrum[c]	0.1 ml	114.0 ± 30.3	13.7 ± 10.0	89.9 ± 7.2
Allethrin	0.1 ml	126.0 ± 45.0	10.3 ± 3.2	91.6 ± 1.5
Esbiol	0.01 ml	54.3 ± 1.5	5.7 ± 0.6	91.6 ± 1.5
Neopynamin	0.1 gm	132.0 ± 29.6	30.3 ± 25.7	78.4 ± 14.2
Permethrin	0.1 gm	146.0 ± 7.5	32.3 ± 4.0	77.9 ± 1.7
D-*trans*-allethrin	0.05 ml	89.3 ± 30.7	4.3 ± 3.0	95.7 ± 2.3
Repellents				
Deet (75%)	0.1 ml	142.7 ± 106.1	22.0 ± 33.0	91.7 ± 11.1
Dibutyl phthalate	0.1 ml	154.5 ± 22.4	87.5 ± 32.8	38.9 ± 22.9
Dimethyl phthalate	0.1 ml	83.0 ± 25.2	9.3 ± 6.4	89.9 ± 9.5
2-ethyl-1,3-hexanediol	0.1 ml	183.0 ± 43.6	34.0 ± 17.3	80.9 ± 10.0
Indalone	0.1 ml	128.0 ± 14.2	27.0 ± 16.5	79.4 ± 11.3
Mosquito Beater[d]	0.1 gm	107.0 ± 20.8	0.8 ± 1.8	98.9 ± 2.4

[a] Mean ±% standard deviation of sum of biting counts taken at 2 minute intervals for 20 minutes. Five tests per compound for Petroleum Oil Fractions and Essential Oils. Three tests per compound for Pyrethrum, Pyrethroids and Repellents.

[b] Percent protection $= 100 \times \frac{\text{Control Biting Count} - \text{Experimental Biting Count}}{\text{Control Biting Count}}$

[c] Pyrethrum concentrate – 22.1% pyrethrum I and 18.3% pyrethrum II.

[d] 0.32 gm (0.1 gm active ingredients) Mosquito Beater® tested.

exhibiting 90% or better protection are considered to warrant further investigation. Direct comparison of the effectiveness of the 4 classes of test materials is complicated by differences in the purity and amounts tested. Each class of compounds had at least 1 material with 90% repellency.

Of the petroleum oil fractions tested, the material with the high naphthalene content (Table 2) gave the best protection (90.9±7.9%, Table 1). The effectiveness of this material is probably not due to its paraffin, dinaphthene, or alkylbenzene content; the other oil fractions tested had higher concentrations of these materials (Table 2) but exhibited lower activity (Table 1). Naphthalenes or substituted naphthalenes would be likely candidates for the repellency exhibited by this material. The active ingredients of Mosquito Beater® include polymethylated naphthalenes (16%) and naphthalene (4.5%). Hocking and Lindsay (1958) reported repellency for Velsicol AR50, a solvent containing methylated naphthalene. McCulloch and Waterhouse (1947) and Bunker and Hirschfelder (1925), however, reported negative results in limited testing of naphthalene itself as a topical repellent on humans.

Citronellal was the most effective of the

Table 2. Composition of petroleum fractions evaluated for area repellency in the laboratory against *Aedes aegypti* (L).

Composition*	Weight Percent	Boiling Points
High naphthalene content (Chevron #78R-3371)		205–344°C (400–650°F)
Substituted naphthalenes	21	
Paraffins	20	
Dinaphthenes	19	
Benzonaphthenes	13	
Higher ringed aromatics	12	
Alkylbenzenes	11	
Sulfur compounds	5	
High paraffin content (Chevron #78R-3372)		205–243°C (400–470°F)
Paraffins	16	
Naphthenes	16	
Dinaphthenes	13	
Benzonaphthenes	4	
Naphthalenes	4	
Alkylbenzenes	3	
High alkylbenzene content (Chevron #78R-3373)		271–305°C (520–580°F)
Alkylbenzenes	100	
High naphthene content (Chevron #78R-3374)		188–260°C) (370–500°F)
Paraffins	41	
Naphthenes	33	
Dinaphthenes	24	
Alkylbenzenes	2	
Naphthalenes	1	

* Approximate composition determined by high mass spectrometry.

essential oils tested, (95.9±5.1% Table 1) for area repellency; geraniol also exceeded 90%. While numerous reports on the activity of essential oils exist in the literature, few of these studies examined the area repellent activity of these compounds. Christophers (1947) and Sarkaria and Brown (1951), using olfactometers, found citronellal to be the most effective material tested for repelling mosquitoes at a distance.

Three of the pyrethroids tested for area repellency exhibited over 90% protection, i.e., D-*trans*-allethrin (95.7±2.3%), esbiol (91.6±1.5%), and allethrin (91.6±1.5%) (Table 1). Most of the area repellency testing of pyrethrum and pyrethroids has been restricted to examining the effectiveness of these materials in closed environments, such as rooms or huts. Several authors (Glynne Jones and Sylvester 1966, Smith and Chadwick 1964) have examined the repellent activity of pyrethrum-containing sprays. Smith and Chadwick (1964) using a synergized pyrethrum reported a residual repellent action for at least 1 month in an experimental hut. The more common use of pyrethrum and selected pyrethroids as area repellents is in slow burning mosquito coils which produce a smoke containing the vaporized active ingredient (Maciver 1963, Wright and Burton 1969). Fales et al. (1968, 1971) reported that the repellent activity of pyrethrum was over five times that of allethrin (1968) and twice as effective as *trans*(+)-allethrin (1971), against *Culex pipiens*. Winney (1969) concluded that neopynamin was significantly less effective than pyrethrum in knockdown and kill. Our tests, in which the materials were not vaporized by heat, showed D-*trans*-allethrin to be more effective than pyrethrum, even though half the volume of test material was used (Table 1).

Testing esbiol at the 0.1 ml level resulted in drastically reduced mosquito activity. The average control biting count for 4 tests was 11.0±7.0. Mosquito activity was still reduced when testing 0.01 ml of esbiol, which had the lowest control biting count of the materials listed (Table 1, 54.3±1.5). This reduction in activity was not observed with any of the other compounds tested. The average 24 hr mortality for mosquitoes tested against 0.01 ml esbiol was 3.0 mosquitoes, the average for all compounds tested was 2.9±1.7.

Mosquito Beater^R was the most effective area repellent of the materials tested (98.9±2.4% protection, Table 1). Means (1973) reported that Mosquito Beater^R was highly effective up to 24 hr after application in lowering landing rates of *Aedes* and *Anopheles* mosquitoes and moderately effective for 24 hr in repelling

black flies (*Simulium jenningsi*) from the area treated. Treatment of a 15 ft (4.6 m) border within the perimeter of a 75×75 ft (22.9×22.9 m) area produced a 90–97% reduction in the mean landing rate 24 hr after treatment (Means 1978). In both studies, 4.5 lb (2.0 kg) of the active ingredient was used for each acre (4,046 m^2).

Deet (75%) was the only standard topical repellent tested that exceeded 90% protection (Table 1) in our studies. This material was tested as a space repellent in mud and grass dwellings where it significantly altered host finding, resting and feeding behavior of anopheline and culicine mosquitoes for up to 3 weeks after treatment (Sholdt et al. 1976).

Simpson and Wright (1967) examined the effects of 2-ethyl-1,3-hexanediol on *Ae. aegypti* and *An. quadrimaculatus;* they reported that 0.5 to 5 ppm of this material blocked the excitation response to carbon dioxide exhibited by these mosquitoes. They suggested the use of area treatments to render biting insects less capable of responding to emanations from persons or animals in the treated area. In our tests, 2-ethyl-1,3-hexanediol exhibited 80.9±10.0% protection (Table 1).

Gorham (1974) tested selected materials for area repellency against mosquitoes in Alaska. The four candidate repellents (benzyl benzoate; N,N-dimethyloctanamide; tertiary-butyl sulfenyldimethyldithiocarbamate; 2-(p-methoxybenzyl)oxy - N, N - dipropylacetamide) were applied to vermiculite and spread over 0.5 or 1.0 acre (2,023 or 4,046) m^2) plots. These chemicals showed no appreciable repellency to mosquitoes. None of the materials tested by Gorham (1974) was examined in our study.

Materials which exhibited greater than 90% protection will be subjected to further laboratory testing. These include citronellal, geraniol, allethrin, esbiol, D-*trans*-allethrin, deet, and Mosquito Beater[R]. Studies have been initiated to identify the active ingredient(s) in the high naphthalene content petroleum oil fraction. Materials which prove most effective in laboratory tests will be field tested for efficacy and duration.

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Selected List of Abbreviations and Symbols

Used in *Mosquito News*

acre. . .spell out
about (*circa*). . .ca.
active ingredient. . .AI
and others. . .et al.
average. . .avg
centimeter. . .cm
compare. . .cf.
cubic centimeter. . .cc
cubic foot. . .ft^3
cubic meter. . .m^3
cubic millimeter. . .mm^3
cubic yard. . .yd^3
diameter. . .diam
dosage mortality. . .DM
dozen. . .doz
emulsifiable concentrate. . .EC
feet per second. . .ft/sec
figure (illustration). . .Fig.
fluid ounce. . .fl oz
foot or feet. . .ft
gallon. . .gal
gram. . .g
granules, granular. . .G
hectare. . .ha
hour. . .hr
inch. . .(spell out if it precedes in). . .in.
kilogram. . .kg
kilometer. . .km
liter. . .spell out
meter. . .m
mile. . .mi.
miles per hour. . .mph
milligram. . .mg
milliliter. . .ml
minute. . .min
number. . .no.
ounce. . .oz
per (with numerals). . ./
percent. . .%
pound. . .lb
pounds per square inch. . .psi
quart. . .qt
relative humidity. . .RH
second. . .sec
significant at 1% level. . .**
significant at 5% level. . .*
square centimer. . .cm^2
square inch. . .$in.^2$
square mile. . .$mi.^2$
square millimeter. . .mm^2
square yard. . .yd^2
ultra low volume. . .ULV
week. . .wk
yard. . .yd