Techniques

A Spore and Pollen Trap for Use on Aerial Remotely Piloted Vehicles

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ABSTRACT

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A spore trap for use on remotely piloted aircraft was designed, constructed, and tested. The trap was constructed from a servo modified to drive an aluminum trapping drum. The edge of the drum was covered with Melinex tape coated with an adhesive mixture to trap impinging spores. The drum could be rotated to multiple positions in front of a 1.0-mm-wide slit orifice. The trap was controlled from the ground by utilizing an auxiliary channel of the remote controls used to pilot the aircraft. The trap sampled

12-14 L of air per minute at a cruising airspeed of about 72-80 kph. Cost of the trap was less than \$75. The trapping system has been employed to sample spore/pollen densities at various low altitudes over peach and pecan orchards. Densities of spore and pollen samples were compared to those recorded by a Burkard 7-day recording volumetric spore trap located on the ground.

Additional key words: Carya, Cladosporium caryigenum, Cladosporium carpophilum, MADDSAP, peach scab, pecan scab, Prunus.

The sampling of airborne spores is crucial in epidemiological studies designed to determine the spore dispersal patterns and disease potential of aerially disseminated plant pathogens. In addition, aerial dissemination patterns of nonpathogenic spores and pollen are of interest to allergists and pollenologists. Probably the most widely used spore traps are the Burkard 7-day volumetric spore sampler (Burkard Scientific Sales, Ltd., Rickmansworth, Herfordshire, England) and the Kramer-Collins 7-day drum sampler (GR Electric Mfg. Co., Manhattan, KS). Other spore sampling devices have been devised and used for general and specific epidemiological studies. These include the widely used Kramer-Collins 24-hour spore sampler (8), the Hirst spore trap (6,15), and the Roto-rod sequential sampler (12). Several other less widely used instruments have been employed for specific applications (6-8,10,11,13,14,16,17). In addition, plans are now available for a highly useful, inexpensive, home-built spore trap similar to the Burkard trap (1).

Most spore traps employed in fields studies are located either on the ground or at most 1–2 m high. Spore concentrations of foliar pathogens in the canopies of tree crops (especially in large trees, such as pecan or walnut) several meters above ground are difficult or impossible to estimate with conventional spore traps (4). Little spore or pollen sampling has been conducted at low altitudes over tree crops. Inoculum of foliar pathogens of large trees disseminated into higher layers of the air may not be accurately estimated by traps located 1–2 m above the orchard floor. The objective of this study was to develop a spore trap for use on an aerial remotely piloted vehicle (RPV) and to evaluate the potential of the apparatus for sampling airborne spore concentrations at low altitudes over crop canopies.

MATERIALS AND METHODS

General design and operation of RPV spore trap. The aircraft employed in this study was part of the Southeastern Fruit and Tree Nut Research Laboratory MADDSAP (Microbial Agent Dispensing Drone for Suppression of Agricultural Pests) program.

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The MADDSAP aircraft employed in the study, originally designed and constructed by the authors to aerially disseminate fungal, bacterial, and viral entomopathogens in ultra-low volumes over large areas of orchard canopies (Fig. 1), was adapted for trapping spores and pollen. The aircraft was also equipped with several additional systems including those for spraying, electrostatic dusting, oil/pesticide volatilization, and insect collecting. The airframe was a modified biplane with fuselage length of 2.04 m, wing dimensions 2.44 × 0.406 m (upper) and 2.29 ×0.406 m (lower), constructed of veneered plywood, spruce, balsa, and Fiberglas and covered with mylar plastic. The engine was a modified two-cycle 50-cc chain saw motor (Quadra 50, Trinden Mfg. Ltd., Huron Park, Ontario, Canada) that operated on a mixture of regular gasoline:2-cycle engine oil (36:1, v/v). Maximum horsepower was 4.0 at 8,000 rpm. The propeller, which was 55.88 cm (22 in.) long, had a pitch of 20.32 cm (8 in.) and developed a thrust of 11.6 kg (25.5 lb). Weight of the RPV ranged from 28.2 to 32.9 kg depending upon the amount of fuel being

Two spore traps (one under each lower wing of the aircraft) were intermittently activated and deactivated by remote control transmitter from the ground. Each trap was located about midway between the fuselage and the wing tip (Fig. 2). The traps were driven by a 1,200 milliampere-hour battery contained in the RPV fuselage. The traps utilized the continuous flow of air beneath the wing of the moving RPV to force spore/pollen laden air into the trap. Thus, in contrast to conventional traps, fans and motors were not required to draw air into the trap (1,7-9,13).

Modification of RPV airframe. Little modification to the airframe was required for installation of the spore traps. The lower wing covering was removed and 60-cm three-stranded wire servo leads were installed from the fuselage through the wing ribs to the points of spore trap attachment. Hardwood blocks $(2.54 \times 2.54 \times 1.25 \, \text{cm})$ were then cemented to the framing. Holes were drilled in the blocks and die-threaded to accept $0.64 \, \text{cm}$ (¼ in.) \times 20-mm nylon bolts for mounting the traps. Veneer plywood $(0.31 \times 3.81 \times 7.62 \, \text{cm})$ was cemented between the wing ribs, flush with the lower wing surface at the point of trap attachment. The servo lead end plug was cemented into a slot in the plywood veneer. The heat-shrinkable mylar covering was then replaced.

Trap housing. A form for molding the plastic trap housing was devised as follows. A teardrop-shaped mold $12.8 \times 6.1 \times 6.0$ cm (flat on one side) was carved from wood and a slightly larger matching hole was cut in a 1.58-cm piece of plywood. Material for



Fig 1. MADDSAP-1 remotely piloted vehicle collecting spores and pollen approximately 15 m altitude above a peach orchard. The arrows show the positions of the pair of aerial spore/pollen traps.

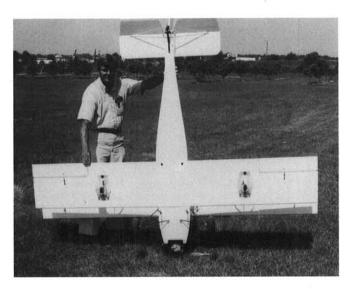


Fig. 2. Underside of MADDSAP-1 aerial remotely piloted vehicle showing position of the pair aerial spore/pollen traps, each one midway between the fuselage and wing tips.

the housing was 0.16-cm-thick clear butyrate plastic sheeting. The sheet of plastic was heated in a lab oven to about 180 C. The softened sheet was then placed on top of the wooden form and the matching plywood form was used to press and stretch the plastic sheet downward over the mold to achieve the desired shape. Excess sheeting at the edges formed a flat outer flange at the bottom of the formed housing. The plastic housing was then cooled with cold tap water while still on the wooden form. A 16-mm-thick aluminum collar was cut to fit over the housing flange and excess housing

flange was trimmed to the dimensions of the aluminum collar. An $(2.0 \times 9.0 \times 0.16 \text{ cm})$ aluminum plate and a matching piece of plastic butyrate were cut and attached to the leading edge of the aluminum collar with small brass hinges. Two 6.4-mm-diameter holes were drilled in the small aluminum plate and one hole in the trailing edge of the aluminum collar corresponding to the hardwood attachment blocks in the wing. The housing and collar were then mounted on the wing with three 6.4-mm nylon bolts (Fig. 3a). The hinge assembly thus allowed the drums to be changed without completely removing the trap from the wing (Fig. 3b). After the housing assembly was fitted to the wing, it was removed and silicone sealant was applied between the housing flange and the aluminum collar, and between the housing flange and the wing. The silicone was kept free of the wing by a wax paper sheet between the silicone and the wing. The housing and collar were then securely mounted on the wing to form a silicone gasket. After the silicone had dried, the wax paper was removed. Two such housings were constructed.

Servo and drum assembly. Two servos (KPS-28; Kraft Systems, Inc., Vista, CA) were modified to rotate 180 degrees. A servo was positioned on the inside bottom of the plastic housing and secured with the servo case bolts through holes drilled in the housing (Fig. 4). The servo lead was secured to the housing wall with tape.

The trap drums were turned on a metal lathe from 7.6-cm-diameter aluminum stock. Finished drums were 5.4 cm in diameter by 1.6 cm thick. The bottom of the drums were recessed about 1.2 cm to remove excess weight, accommodate the top of the servo head, and reduce the depth of the trap. A hole was drilled in the center of each drum to accept the threaded servo spindle. An alignment screw was set into the recessed side of the drum to match a retaining hole in the servo head. The drum was held in place with a knurled thumb-nut on the threaded servo spindle (Fig. 4).

Orifice assembly. The orifice was constructed from a piece of 1.1-cm-diameter × 4.0-cm-long brass tubing. One end of the tube

was compressed and adjusted via a thickness gauge to an orifice slit width of 1.0 mm. Final slit size was 15 mm long × 1.0 mm wide. An 11-mm-diameter hole was cut in the leading surface of the plastic housing in line with the cylindrical side of the drum. The brass orifice tube was inserted through the hole in the housing with its axis aligned radially at the midline of the face of the drum and the 1.0-mm slit was positioned parallel to chords of the drum and 1 mm from its surface. The brass orifice tube was secured in this position with epoxy putty. A 1.0-cm-diameter hole was also cut in the trailing edge of the housing to allow air flow out of the trap housing (Fig. 4).

Preparation, removal, and examination of the spore trap tape. The ends of the Melinex tape (1.3 cm wide) were affixed to the trap drum with double-faced tape. The Melinex tape surface was then coated with 10% polyvinyl alcohol in distilled water and dried in an oven at 50 C for 15 min. After cooling, the Melinex tape was recoated with Vaseline petroleum jelly plus 10% paraffin thinned to a soft paste with toluene. The drum and tape were then gently reheated on a hotplate until all brush streaks disappeared. The readied drum was cooled before installation in the spore trap.

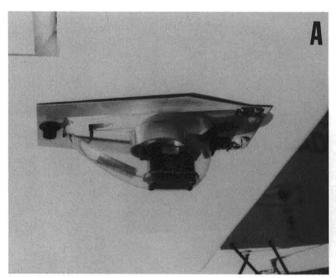
After aerial spore sampling, the tapes were removed from the drums, mounted on microscope slides and stained with acid fuchsin-lactophenol (Fig. 5). Mounts were made permanent by incorporating 1% polyvinyl alcohol into the stain. Spore/pollen bands on the trapping tapes were examined at ×400 with phase-contrast optics (Fig. 5a). Spores were identified by staining characteristics, size, and shape. Spore counts were made by counting all spores in the trapping bands.

Trap electronics and operation. The servo leads from each trap were joined inside the fuselage with a Y-harness having a "glitch"eliminator or spike-reducing choke in line to reduce extraneous radio frequency interference. The Y-harness was then connected into an auxiliary channel port of the RPV receiver in the fuselage. The spore traps were operated in flight by a slide switch on the transmitter (KPT7C IV Airborne Pack; Kraft Systems, Inc., Vista, CA) that controlled the auxiliary channel. The slide switch was marked for 20 positions. Movement of the slide switch to each position rotated the spore trap drum to each of 20 sequential sampling positions. The first sequential position was reserved for takeoff, climb out, descent, landing, altitude changes, or other times when trapping was undesirable. After the RPV attained the desired altitude and was trimmed for level flight, the drum was rotated to one of the remaining 19 positions and sampling was conducted for a specific length of time. When sampling was concluded at this altitude or sampling area, the drum was returned to the first position and the RPV was flown to the next altitude or sampling area. The drum was then rotated to a different position, etc. Although returning the drum to the first position caused previous spore bands to pass by the collection orifice, the drum rotated very quickly; therefore, return to the starting position was nearly instantaneous and very little, if any, contamination of previous spore bands resulted.

Air flow tests. Air flow tests were conducted by securing the trap to a mock wing section, removing the drum, and positioning the probe of a thermo-anemometer (type 8500; Alnor Instrument Co., Niles, IL) through a hole in the mock wing just behind the slit orifice. The wing was then suspended outside the window of an automobile driven at speeds approximating that of the MADDSAP RPV in straight and level flight (70–90 kph). Airflow calculations were made utilizing slit orifice cross sectional area and automobile speed. Orifice slit width was adjusted until the desired air flow was obtained. In flight, it did not matter if the RPV was moving into, with, or at an angle to the wind; its speed in relation to the air mass it flew through was constant. Therefore, the rate of airflow through the spore traps was also constant and did not vary from the desired flow rate of 12–14 L/min.

Field tests. Field tests were conducted over a 13-hectare (ha) orchard of 55-yr-old pecan trees, Carya illinoensis Koch 'Schley' and 'Stuart,' and over a 1.2-ha orchard of 9-yr-old peach trees, Prunus persica (L.) Batsch 'Coronet.' Heights of pecan and peach trees were 19.0-22.8 m and 2.5-3.0 m, respectively. A Burkard

volumetric spore trap, adjusted to an airflow of 11 L/min, was placed on the orchard floor in each orchard for concomitant collections. Aerial sampling was conducted at three altitude ranges over pecans 26-29, 35-38, and 50-57 m and three altitudes over peach 6-9, 15-18, and 30-37 m. The air layer at each altitude was sampled for 5.0 min utilizing a different position of the spore trap drum (Fig. 5a). Aerial samples were taken during the early evening on three separate dates. Spores were identified to genus level where



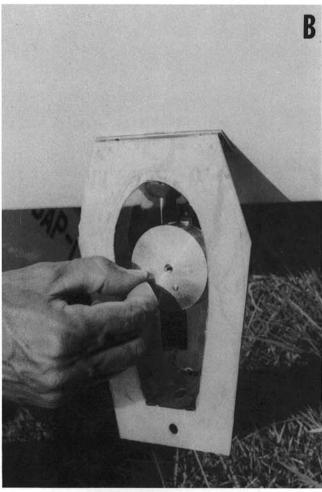


Fig. 3. Closeup of one of the aerial spore/pollen traps mounted on bottom of the lower wing of MADDSAP-1, an aerial remotely piloted vehicle. A, Mounted for collection. B, Rear nylon bolt removed and trap tilted down on front hinges for access to trapping drum for easy changing.

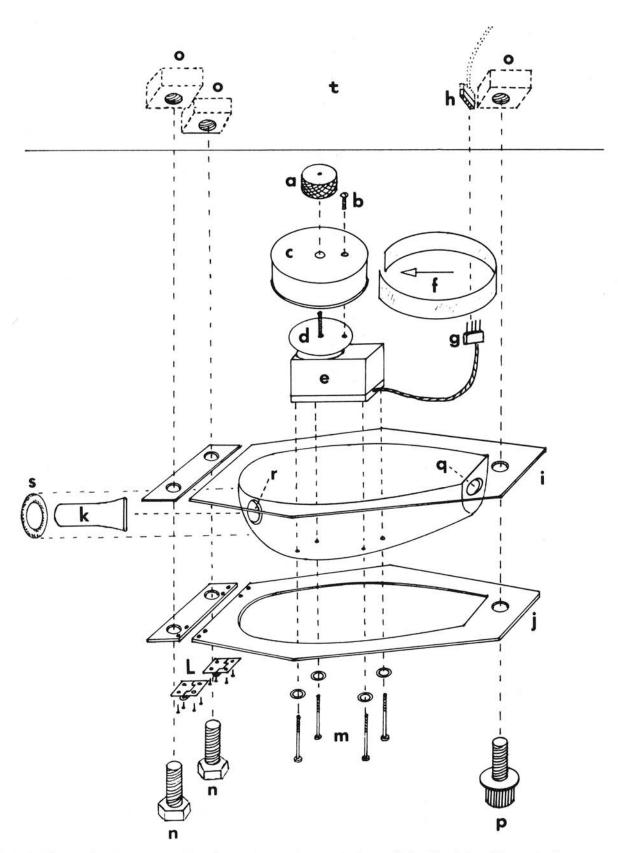


Fig. 4. Exploded diagram of aerial spore trap designed for use with an aerial remotely piloted vehicle. a) Knurled retaining nut, b) alignment screw, c) spore trap drum, d) servo control plate, e) servo, f) clear Melinex tape coated with adhesive, g) servo lead and male jack, h) female servo jack embedded in wing surface, i) molded plastic spore trap housing (canopy), j) aluminum housing collar, k) orifice tube, l) small brass hinges, m) servo case bolts and washers, n) nylon spore trap mounting bolts, o) taped hard-wood blocks embedded in wing bottom, p) nylon removable thumb-bolt, q) rear air vent hole in spore trap housing, r) orifice tube hole, s) epoxy putty to secure nozzle in housing, t) under-surface of bottom wing.

possible. Spore trap tapes from the Burkard trap were examined for the hour corresponding to the time of RPV trapping. Data of spore and pollen density from both aerial and Burkard traps were then adjusted to density of spores/pollen per cubic meter of air sampled. Samples for the two aerial traps were combined.

RESULTS AND DISCUSSION

Air flow tests. A slit width of about 1.0 mm corresponded to a

calculated air flow of 12–15 L/min at an air speed of about 72–80 kph (45–50 mph). Since the cross sectional area of the rear hole exceeded the cross sectional area of the orifice slit, the vacuum caused by the aerodynamic shape of the trap housing aided in pulling air through the housing.

Field tests. Aerial spore/pollen catches at various altitudes over orchard canopies compared to a Burkard trap located on the orchard floor revealed the unique relationship of aerial spore

TABLE 1. Frequency of airborne spore and pollen species at various altitudes below and above a pecan orchard

	11 May Time EST				16 May Time EST				24 May Time EST			
	1820- 1910	1834- 1839	1840- 1845	1845- 1850	1807- 1827	1807- 1812	1816- 1821	1822- 1827	1821- 1850	1820- 1825	1833- 1838	1839- 1844
	Altitude (m)				Altitude (m)				Altitude (m)			
·	0.5ª	26-29 ^b	35-38 ^b	50-57 ^b	0.5ª	26-29 ^b	35-38 ^b	50-57 ^b	0.5ª	26-29 ^b	35-38 ^b	50-57 ^b
Cladosporium												
caryigenum	25,604	431	284	293	11,856	438	800	131	15,542	3,958	6,761	3,804
Other Cladosporium sp.	205	138	77	300	129	46	39	62	205	531	501	847
Alternaria sp.	1,223	92	139	31	1,284	54	131	46	3,131	847	1,601	639
Epicoccum sp./										0.17	1,001	039
Stemphylium sp.	1,489	100	92	77	281	8	31	15	737	169	193	162
Cercospora sp./	100 100 000				(1557)		5.5		2.50	107	195	102
Cercosporidium sp.	53	46	15	23	38	8	0	8	30	23	31	8
Helicoma sp./										23	31	.0
Helicosporium sp./												
Helicomyces sp.	8	23	8	31	0	0	8	0	137	231	285	539
Helminthosporium sp./					88	- 5				231	203	339
Bipolaris sp.	15	8	0	8	38	0	0	0	61	85	139	100
Dirondion sp.	0	0	0	8	0	0	0	0	0	0	0	0
Trichothecium sp.	0	54	15	69	0	15	8	39	190	385	693	154
Cylindrocladium sp.	0	39	0	0	0	15	0	0	53	77	231	0
Misc. ascospore Octets	0	62	62	123	0	0	0	0	8	85	39	15
Misc. fungal species	2,188	12,743	7,561	3,534	2,143	1,848	9,979	4,974	6,597	15,808	18,434	21,028
Carva illinoensis		STEPPINGER	1075773	K03550		17.7	-15.55		-14.6	. 5,000	10,434	21,020
(pecan) pollen	106	85	152	138	0	46	85	54	0	0	0	0
Misc. pollen	8	146	169	46	114	254	288	177	53	254	339	331

^aSpore-pollen catches from Burkard volumetric spore trap located on the orchard floor reported as spores-pollen per cubic meter of air.

TABLE 2. Frequency of airborne spore and pollen species at various altitudes below and above a peach orchard

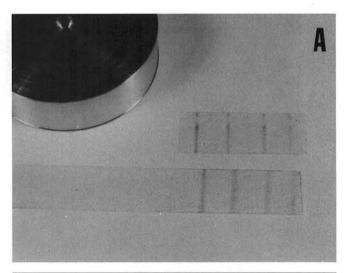
	11 May Time EST				16 May Time EST				24 May Time EST				
	1938- 2006	2005- 2010	2000- 2005	1949- 1954	1831- 2010	1950- 1955	1926- 1931	1908- 1913	1922- 2030	2009- 2014	1949- 1954	1929- 1934	
	Altitude (m)				Altitude (m)				Altitude (m)				
	0.5ª	6-9 ^b	15-18 ^b	30-37 ^b	0.5ª	6-9 ^b	15-18 ^b	30-37 ^b	0.5°	6-9 ^b	15-18 ^b	30-37 ^b	
Cladosporium													
caryigenum	1,302	362	631	208	2,865	1,394	639	616	8,314	3,835	2,834	4,381	
Other Cladosporium sp.	68	131	116	308	46	15	54	39	84	408	732	616	
Alternaria sp.	821	77	46	116	532	154	131	77	424	678	832	778	
Epicoccum sp./											(APATIES)	1,000	
Stemphylium sp.	1,596	39	92	108	243	69	216	69	46	131	239	231	
Cercospora sp./											2000	17710	
Cercosporidium sp.	30	0	0	39	15	0	15	0	84	54	8	23	
Helicoma sp./ Helicosporium sp./													
Helicomyces sp.	15	8	8	0	8	15	8	8	175	354	339	516	
Helminthosporium sp./								1.00	15.0.61			0.10	
Bipolaris sp.	15	0	0	0	8	23	0	31	30	54	108	216	
Dirondion sp.	0	0	0	0	0	0	8	0	0	15	8	0	
Trichothecium sp.	30	62	62	62	15	8	15	15	61	85	31	162	
Cylindrocladium sp.	46	46	15	46	0	8	8	8	8	85	0	0	
Misc. ascospore Octets	0	0	0	0	0	8	0	0	15	23	8	0	
Misc. fungal species	920	13,781	13,614	8,586	2,295	3,203	2,025	1,863	8,428	17,826	22,438	20,613	
Carya illinoensis				7.5		717	-				,		
(pecan) pollen	68	597	292	362	0	69	62	39	0	0	0	0	
Misc. pollen	15	123	169	123	68	431	447	354	46	400	239	316	

^{*}Spore-pollen catches from Burkard volumetric spore trap located on the orchard floor reported as spores-pollen per cubic meter of air.

Spore-pollen catches from aerial RPV traps at various altitudes above the orchard canopy reported as spores-pollen per cubic meter of air.

Spore-pollen catches from aerial RPV traps at various altitudes above the orchard canopy reported as spores-pollen per cubic meter of air.

distribution to altitude for several fungal genera. The frequencies of airborne spores and pollen species trapped via aerial RPV spore traps over pecan and peach orchards are reported in Table 1 and Table 2, respectively, and compared to Burkard volumetric traps located on the orchard floors. Meterological conditions during the flights are reported in Table 3. During the spring of 1984, a moderate foliar epidemic of pecan scab, caused by the fungus Cladosporium caryigenum (Ell. et Lang.) Gottwald, occurred in the pecan orchard under study. Table I shows the airborne concentration of spores at 0.5 m under the orchard canopy. A surprisingly large number of spores of C. caryigenum were also trapped at altitudes of 50-57 m above the canopy. C. caryigenum can be separated from other Cladosporium species by conidial morphology. Intact chains of blastoconidia of C. caryigenum were often observed on Burkard spore trap tapes from previous studies. Chains of conidia are frequently liberated from the infected leaf surface intact. Neither impact on Burkard or RPV spore trap surfaces appeared to cause breakup of such chains (Fig. 6) (3). Whether spores at this altitude were produced and released from



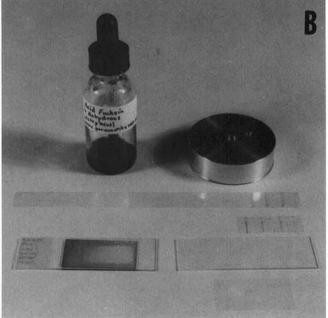


Fig. 5. Spore trap drum and Melinex tape dissection. a, Spore trap drum with Melinex tape removed showing bands of trapped spores and pollen on tape. b, Once the tape is removed from the drum, the segment(s) with trapping bands on them are cut away and mounted on a microscope slide with an acid fuchsin/lactophenol mountant and sealed with a 22×40 - or 22×60 -mm cover glass.

the orchard under study was not determined. However, these findings demonstrated for the first time a mechanism for long-distance dispersal of inoculum between orchards (2,4,5). Other Cladosporium species were often found in higher concentrations at higher altitudes than within the canopy. The same could be said for most spore types which were identified. Alternaria, Stemphylium, Epicoccum, Cercospora, and Cercosporidium species are common saprophytes and/or pathogens of grasses and other weed species on the orchard floor. Their aerial concentrations were usually much higher at 0.5 m than at any of the three altitudes above the orchard.

Pollen shed from pecan (an amentiferous species) was observed during the first week of May then began to decline in the orchard under study. The RPV spore traps demonstrated that pollen concentrations during pollen shed periods at altitudes above the canopy and near the orchard floor were nearly equal. During post-pollen-shedding periods, pollen was encountered more often above the canopy than near the orchard floor. This pollen may have originated from later-pollinating cultivars in the general area or may simply have not yet been cleansed from the air. Pollen from other angiosperm species was also always encountered in greater numbers above than within the pecan orchard canopy.

During late spring of 1984, a moderate epidemic of peach scab caused by *Cladosporium carpophilum* Thum. occurred in the peach orchard under study. The buildup of disease and inoculum production corresponded to the time during which the aerial spore sampling was conducted (Table 2). Fairly high numbers of spores of *C. carpophilum* were present at altitudes up to 30–37 m (28–35 m above the canopy). These findings substantiate the recently

TABLE 3. Meterological conditions during duration of spore-trapping flights

	11 May 1820–2010	16 May 1807–2010	24 May 1821-2030
Temperature (C)	25-20	24-20	27-24
Relative humidity			
(%)	38-52	33-52	52-72
Wind speed (kph)	1.86-1.24	1.24-1.86	1.86-0.31
Wind direction	E-NE	N-NW	S-SE
Rain (cm)	0	0	0
Cloud cover	Clear	Clear	Clear
Solar radiation			
(avg. per day) J/m ²	2.636×10^{7}	2.605×10^{7}	1.353×10^{7}
Barometric pressure			
(cm) (ground)	76.49	76.45	76.40

^a All meterological readings were taken at the Byron, GA, weather substation about 200 m from the orchards under study.

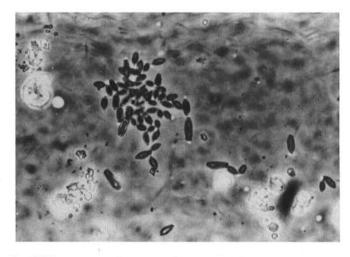


Fig. 6. Phase contrast micrograph of spores of *Cladosporium caryigenum* collected with an aerial spore/pollen trap mounted on an aerial remotely piloted vehicle. Stained with acid fuchsin in lactophenol (×420).

reported conclusion that although spores of *C. carpophilum* are splash dispersed they are also subject to aerial discharge and dissemination (5,9).

Most other fungal species that were encountered followed the same altitude gradation pattern over the peach and pecan orchards. In general, airborne spore concentrations of most of the fungal species encountered increased as the spring progressed.

The RPV spore traps were employed only at relatively low altitudes where altitude could be easily estimated. Although the RPV is capable of attaining altitudes of several thousand meters, altitude estimation becomes a problem due to lack of reference. An inexpensive means of altitude radio telemetry is being sought to solve this problem. However, at lower altitudes or even at high altitudes where accuracy of altitude estimations is not critical, the RPV spore traps can be of considerable use in estimating spore/pollen populations and in investigating aerial inoculum dispersion and dissemination. Such data have not been easily obtained previously.

The two traps attached to the aircraft provided good balance for the RPV during flights, and allowed simultaneous replication of samples. The spore traps were found to be easily and quickly serviceable during and after trapping operations.

Although a sophisticated drone aircraft with costly electronics was utilized in this study, relatively inexpensive one-quarter scale model aircraft could also be used. Most of the radio controls and necessary hardware for such models are available through local hobby stores for a total systems cost of \$700-1,000. Each trap cost less than \$75, but this amount could be decreased considerably by employing less expensive servos.

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