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CULEX ANNULIROSTRIS BREEDING SITES IN URBAN AREAS: USING REMOTE SENSING AND DIGITAL IMAGE ANALYSIS TO DEVELOP A RAPID PREDICTOR OF POTENTIAL BREEDING AREAS

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ABSTRACT. A rapid technique is being developed and assessed to identify urban breeding sites of *Culex annulirostris,* which is a vector of an arbovirus (Ross River virus). Field survey and laboratory identification were used to identify breeding sites for the species. Ephemeral sites became the focus of the research and other mosquito species were recorded. The sites were located on digitized images of 1: 30,000 color aerial photographs. Training sites were used to create "themes" for the main ephemeral breeding sites and the MicroBRIAN image processing package was used to map the themes to each image. The accuracy and completeness of identification were evaluated with reference to the original field site identification and by further field checks. The accuracy was 87% and the completeness was 75%.

Arbovirus diseases such as Ross River virus (RR) and Barmah Forest virus are increasing problems in Queensland, Australia (Dale 1994). Attention has in the past focused largely on the intertidal salt marsh-breeding vector, *Aedes vigilax* (Skuse). Recently, however, interest has grown in some freshwater mosquito species that appear to play a major role in the transmission of arbovirus disease. One such species is *Culex annulirostris* (Skuse), which is found throughout mainland Australia and which is a known vector of RR virus. It occupies a variety of habitats under the broad heading of freshwater wetlands, with some breeding in brackish or nutrient-rich waters (Marks 1982, Kay and Aaskov 1989, Russell 1993). These habitats may be scattered, be of relatively small size, and may go unnoticed in and around urban areas where they pose the greatest human health risk. However, the habitats are amenable to rapid identification from remotely sensed data, provided that the relevant environmental variables have been identified. For control to be effective it is essential that a very high proportion of the breeding sites are known. Remote sensing analysis has the potential to provide the comprehensive cover required.

Studies have indicated that there may be a relationship between the spatial distribution of mosquito immatures and environmental variables at and around the breeding sites producing immatures (Pierson and Morris 1982, Morris et al. 1990, Morris and Robinson 1994). Characteristics such as water, wetlands, and associated vegetation may be readily identified from remotely sensed data and this has application for rapidly and cost-effectively identifying potential breeding sites. The potential of remote sensing and geographic information systems has been generally described by Hugh-Jones (1994), Ritchie and Dale (1994), and, for a range of arthropod vectors, by Washino and Wood (1994). Dale et al. (1986) showed that breeding of *Ae. vigilax* in salt marshes in southeast Queensland is related to particular habitat characteristics that can be identified from large-scale aerial photographs.

This note summarizes the results of a pilot project whose objective was to develop a system to rapidly, comprehensively, and cost-effectively survey and predict potential wetland sites as small as 100 m^2 for the breeding of *Cx. annulirostris* and other freshwater mosquitoes that are or may be vectors of arbovirus disease. This study is based on remote sensing and automated image analysis.

Color aerial photographs at a scale of 1: 30,000 taken in April 1993 were available from the Brisbane City Council (BCC), with enlargements at 1:10,000 for field use. These photographs had not been taken under optimum (wet) field conditions, because there had been no rainfall in the preceding 9 days and only 1.8 mm in the preceding 18 days (Australian Bureau of Meteorology). To provide a range of habitat types, 2 areas were selected in metropolitan Brisbane, in southeast Queensland. One area was coastal with low-density suburbs and the other was further inland and highly urbanized. Likely breeding sites were identified visually on the aerial photographs and then were located and checked on the ground. During ground checking other sites that appeared to be breeding areas were also included and marked on the aerial photographs and on the standard 1:25,000 road maps used by BCC officers for site location.

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During the months of February and March 1995, while surface water was present, 78 sites were checked. Only wet sites were classified and numbered, regardless of the presence of mosquito larvae or pupae.

The strategy of the project was modified after a prolonged dry spell (little or no rain for 20 days) made it impossible to identify and classify potential temporary-water breeding sites. Major macrohabitat types with the potential for mosquito breeding were numbered and categorized in a small part of the coastal area. This assessment was based on the terrain and whether it was likely to contain depressions that would hold water for sufficient time for mosquitoes to develop and emerge. Because the focus was on ephemeral sites, we recorded mainly grassy fields with uneven terrain and other grassy breeding sites such as natural and seminatural drains and their margins, as well as wet forest sites (vegetated mainly with *Melaleuca* spp.).

A Garmin Surveyor 2 Global Positioning System (GPS) was used to record the Australian national grid coordinates of each site to facilitate integration with the BCC geographic database. Using a 3-min average, the reading was generally accurate to between 3 and 10 m. Larvae were collected in the field at each site using standard dipping procedures. Samples consisting of 20–40 dips in a 100-m^2 area were collected for laboratory sorting, counting, and identification. The habitat survey focused on identifying the characteristics of the sites and their surroundings. The characteristics observed were limited to those that are relevant to mosquito ecology and identifiable on aerial photographs at a scale of 1:10,000. Variables included site type, bottom characteristics, amount of shade, aquatic vegetation, and terrestrial vegetation. Data were tabulated, and because they were frequencies, chi-square analysis was used to determine if there were significant relationships between mosquitoes and their environment.

Transparencies of the standard 1:30,000 color aerial photographs were digitized using the Image 100 (IMAGELAB) package to produce a 3-layered image file in red, green, blue (RGB) format. This was transformed for use by the MicroBRIAN image analysis system (Jupp et al. 1985). The pixel size was 10 × 10 m. For each band of the image spectral values outside the first and last percentile were excluded and the remainder, between 1 and 99%, were "stretched" to cover the full range of 0–254. This was done to enhance contrast. Both actual and potential mosquito breeding sites that were positively identified in the field were located on the image and used to create training themes in the image-processing package. A training theme was identified by selecting one or more pixels and ascribing a descriptor to it, usually a name and number, such as "grassy field 1." The spectral characteristics of the pixel(s) were filed and used to select other pixels on the image with similar spectral values in the RGB bands. This was a supervised classification process. The pixels selected were given a distinguishing color and were used to create a map of the whole image. This was printed at a large scale (1:6,500) for field checking.

The results were assessed, using field data NOT used to create the training themes, by calculating accuracy, measured as the percent of breeding sites identified by the image analysis as ones that were ground truthed as actual or potential breeding sites; and completeness, measured as the percent of breeding sites identified in the field as actual or potential breeding sites that were also identified by image analysis.

Brisbane experienced a summer-long drought prior to the start of the project. In January 1995 the rainfall of 43 mm was only 26% of the long-term average. The drought persisted during the project period, with the exception of major rainfall events between February 12 and 16, when 241 mm of rain fell in Brisbane causing considerable flooding. Extensive flooding initially made it impossible to know where specifically to look for larvae. As the dry ground rapidly absorbed the water, the number of potential breeding sites increased. Unfortunately, the ground was so dry that the remaining water was quickly absorbed. Additionally, very little rain fell in the remaining 6 wk of the project so that temporary surface water suitable for breeding by *Cx. annulirostris* remained for only 2 wk and, for the most part, larvae were present only during one of those weeks.

The data analysis indicated that the only significant relationships for *Cx. annulirostris* were with site type (df = 7, χ^2 = 17.25, P = 0.02) and a bare or grassy site (df = 1, χ^2 = 7.51, P = 0.006). The presence of emergent vegetation and the absence of woodland (Spect 1970) in the surrounding area made it somewhat more likely that a site would have *Cx. annulirostris* but this was not statistically significant ($P > 0.05$). Data for the 16 other species were particularly sparse and no significant relationships were found. Thus, *Cx. annulirostris* were found most often in permanent ponds, temporary pools, and depressions in grassy fields and marshes, whereas other species were common in a wider range of habitats and exclusively in drains and containers (Fig. 1). Figure 2 shows a typical grassy field site.

As the area dried out we made subjective assessments over a small area as to the potential

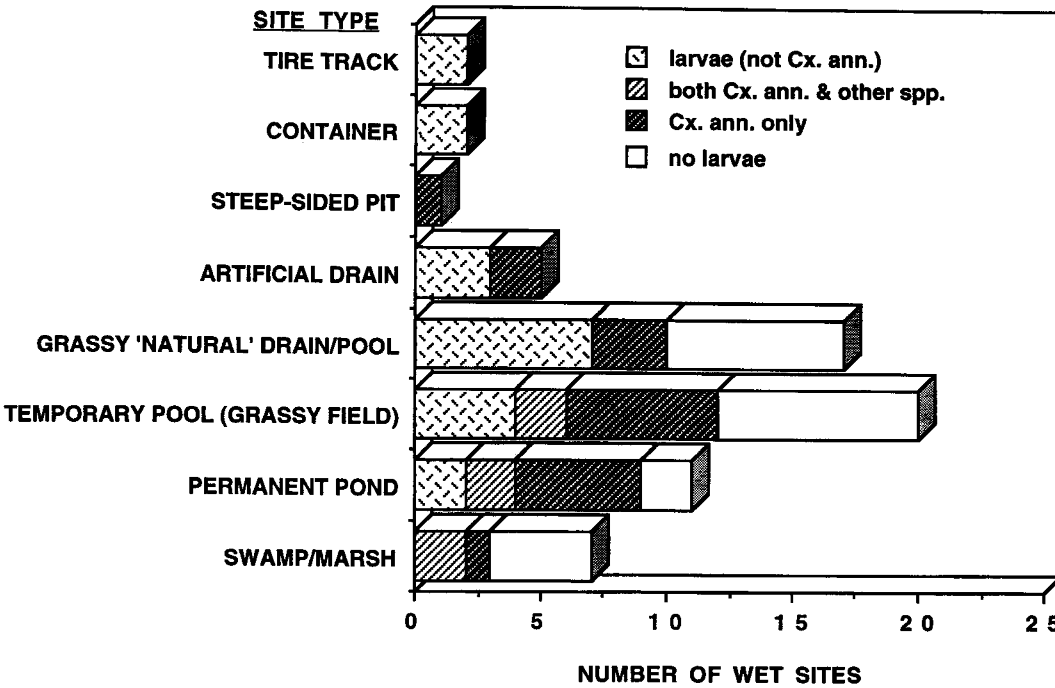

Fig. 1. Distribution of mosquito larvae by site type for wet sites surveyed following rainfall (inland and coastal sites).

of a site for breeding of mosquitoes. Over 90% of these potential sites were associated with grassy habitats of ponds, depressions in fields, and both natural and artificial drains.

The overall results of our surveys suggest that the potential nondomestic mosquito breeding sites in inland Brisbane fall into 3 main categories: temporary pools in flooded grasslands (grassy fields), permanent ponds with floating or emergent vegetation, and major natural or seminatural grassy drains. The latter often had a berm and the adjacent grasslands frequently fell into the grassy field category. This resulted in the numerous but typically small grassy pools that made up the majority of the *Cx. annulirostris* breeding sites that became the main focus of the image analysis. After the initial surge of *Cx. annulirostris*-positive sites, most of the wet sites encountered and classified were drains, a habitat where we did not previously find this species. Despite the presence of still water in many of the drains, we recovered very few larvae of any species from these sites.

For the wet area, and focusing on the grassy fields, we used 2 sites identified in the field (which contained *Cx. annulirostris*) as themes to be mapped on the images of each area. We first experimented with larger numbers of positively identified sites as themes, but found that this resulted in large areas being highlighted as potential breeding areas. This was considered impractical because the objective was to minimize the preliminary field survey in the fully developed method. For both areas the overall results were similar, so they are combined here. Of the 78 sites initially surveyed, 68 (87%) were correctly (accurately) identified by the image analysis; of a sample of 71 sites identified by the analysis as "grassy field," 53 (75%) were confirmed in the field as definite or likely breeding sites (75% completeness).

In the dry area, 2 *Melaleuca* sites identified as potential breeding areas were used as themes for the image analysis. The resulting map initially appeared disappointing as it highlighted much more than the *Melaleuca* forest and woodland. However, field checking showed that, although only 9 (41%) of the 22 sites had *Melaleuca* as a common genus, 16 (73%) sites were wet sclerophyll woodland/forest and likely to produce mosquitoes.

If permanent wetlands act as reservoirs and grassy fields provide population expansion opportunities for *Cx. annulirostris*, it should be possible to control the explosive growth of the species during wet periods by maintaining tight control of the reservoir populations and by vigilance over the ephemeral sites. This requires

Fig. 2. A typical grassy field in the study area.

that every major breeding site within flight range of the human populations being protected is identified and mosquitoes controlled there. This is where a rapid survey tool is vital.

Speedy identification of potential breeding sites can help mosquito control managers focus surveillance and control on those areas most at risk. The method of identification proposed here may well uncover areas hitherto unknown (because sites may be small or not readily accessible) and identify species–habitat associations that were previously not recognized. Thus, the method contributes to increasing the efficacy of mosquito control and helps reduce the incidence of RR virus (or other mosquito-borne disease) and is applicable to other places. There could also be benefits to the routine operation of a vector control unit by integrating the breeding site maps with a geographic information system.

Because many of the mosquito breeding sites were much smaller than the 100-m^2 pixel of the digitized aerial photos, it became apparent that the MicroBRIAN images prepared for this study were not of sufficiently large scale to discriminate the very small breeding sites *per se*. This is true for the small flooded grasslands sites, which were often less than 5 m wide, and for the roadside drains, which were typically less than 2 m wide. Nevertheless, even small depressions that are not visually detected on the aerial photographs can be identified in their larger grassy field context by our image analysis method.

In conclusion, breeding site surveys should be done using aerial photographs taken during optimum mosquito-breeding site surface-water conditions. If aerial photographs are not available for the appropriate time, 35-mm slides or videotape images taken from low-flying aircraft may be used. This has yet to be investigated but is probably the most efficient method. These images can then be reviewed and appropriate areas digitized and used in computer image analysis programs. Large-scale aerial photographs can often be analyzed visually without computer assistance. Although this is effective for identifying relatively large areas, the image analysis is much more subtle in its discrimination of sites and is to be preferred to only visual interpretation.

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