Natural Resources Institute

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[a component of Project A0166]

Overseas Development Administration

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## Introduction

1. The spread of rice tungro virus disease within a rice field is determined by the "vegetative" movement of the leafhopper vectors, particularly *Nephotettix virescens* (Distant) and *N.nigropictus* (Stål). Factors of importance for modelling and predicting disease spread include the range and frequency of flights, and the duration of settlement on individual host plants (Holt *et al.*, 1992).

2. Visual observation of leafhoppers moving in the field is only partially effective, because their small size and their colour make them hard to see, and mechanical disturbance of the crop canopy by an observer is likely to modify their behaviour. Moreover, accurate recording of any observed movement trajectories is difficult without an elaborate co-ordinate system. These already considerable difficulties are compounded by the need to make observations at night.

3. Video equipment has been used successfully to record short-range flight of moths and other insects in the field, during both day and night (Riley, 1993), but the small size of leafhoppers (*c.* 4 mm), and their low contrast when seen against vegetation, make them unpromising candidates for video studies. However, it has been claimed in the literature that when insects of somewhat larger size (tsetse flies) are marked with reflective (Rennison *et al.*, 1958) or fluorescent (McDonald, 1960) materials, and suitably illuminated, the range for visual detection can be extended as far as 10 m, at least when the insects are stationary. This suggests that with a sensitive video camera one might expect to achieve a comparable or even greater detection range for marked leafhoppers, and that quantitative video studies of the short range movements of leafhoppers might therefore be feasible.

4. In this report we describe a short, two week, experimental investigation of the degree to which the visibility of leafhoppers to video can be enhanced by the use of marking materials.

## Background

5. The small size of leafhoppers does not in itself preclude the use of video recording, because with the appropriate choice of lens and viewing distance, even very tiny insects can be resolved. However, size critically effects the *length* of flight track observable. If a lens is chosen to produce the maximum field of view which just allows detection of a target insect at some

selected range, then under ideal conditions, the insect image will register (at any one time) as a point target on just a single line of the 575 used in normal video displays. The maximum straight line flight trajectory in a plane perpendicular to the viewing axis of a stationary camera would then be approximately 575 times an average body dimension in the vertical (screen) direction, and 1.3 times this distance across the screen. For a leafhopper of $\approx$ 4 mm in size, this would give a viewing area of $\approx$ 2.3 x 3.0 m.

6. It is important to note that this coverage could be achieved only if the leafhopper generated a bright image against a dark background. In other, more normal conditions, registration on 10 or more display lines may be required for detection, with a corresponding hundredfold reduction in effective viewing area. Conversely, if a leafhopper forms a *very* bright source against a very dark background, phosphor "flare" in the video camera would make the insect visible over a larger field of view than that calculated above (Riley, 1993).

7. The degree to which video can be used to monitor leafhopper movement is thus seen to be critically dependent on the degree to which it is possible to enhance their *contrast* over the background against which they are seen. Extremely high contrast enhancement of unmarked insects is possible if they can be viewed appropriately illuminated against the sky (Schaefer & Bent, 1984; Riley *et al.*, 1990). However, the very low flight altitude of leafhoppers moving within a crop and the requirement to monitor them when they are settled on plant leaves, preclude this solution, and dictate that some form of marking technique be used.

**Choice of markers to improve contrast against vegetation.**

8. There appear to be two ways in which contrast can be enhanced by markers. The first is to use marking material which is more highly *reflective* than the background, and the second is to use a material which *fluoresces* strongly, preferably at wavelengths not present in background reflections, but to which the observing equipment is sensitive.

*Reflecting materials*

9. The most effective reflecting materials are those which return a high proportion of incident illumination towards the viewer. Specular reflections from a smooth surface or a mirror do this most efficiently, but mirrors are effective only when the angle of reflection is *directly* towards the observer, and so they are not useful as markers on moving targets. However, materials are readily available which concentrate reflected illumination within a small solid angle directed back towards the illuminator, and provided that the viewer is also within this angle, their effective reflectivity far

exceeds that of diffuse surfaces. The dramatic effect of materials of this type is well illustrated by the high visibility of reflective road signs at night. It was therefore decided to evaluate directionally reflective materials as potential markers for leafhoppers.

*Fluorescent materials*

10. Fluorescent materials absorb light in one spectral range, and emit a proportion of the absorbed energy as radiation at lower frequencies. High efficiencies of conversion (up to 95%) are achieved with solutions of the exotic chemicals used in dye lasers, but these materials are usually toxic or carcinogenic, and so are not suitable for use in the field. Less efficient but more common compounds, which absorb in the near ultra-violet part of the spectrum (300 - 390 nm) and fluoresce in the visible, are available as powders in a variety of colours (e.g. Hostasol (Hoechst Ltd.) in yellow and red, Uvitex (Ciba-Geigy Ltd.) in blue/white). These have been frequently used in insect mark-recapture studies in the field (Southwood, 1978), and so appeared to be suitable for assessment as contrast-enhancing markers.

**Possible illuminator wavelengths**

11. In order to use high efficiency directional reflectors as markers, some form of localised artificial illumination is required. It is anticipated that observations of leafhoppers are to be carried out mainly at night, so artificial illumination would be required in any case, for both reflecting and fluorescing markers. The question then arises of which wavelength region to use. Given the time and resources available for this study, choice was in practice limited to illumination sources already held by the Radar Unit, *viz.* near infra-red, visible and near ultra-violet. Potentially more effective illuminating techniques using narrow band, scanning lasers were not within the scope of this short study.

*Near infra-red*

12. Earlier nocturnal video studies of insect flight have used illumination in the near infra-red (670 - 1500 nm), for the very good reason that this wavelength region appears not to perturb the behaviour of illuminated insects (Riley, 1993). Unfortunately, vegetation reflectivity rises from about 5% at 600 nm to 30 - 50% at near infra-red wavelengths (Monteith, 1973), and the higher the reflectivity of vegetation, the lower the contrast achievable for a marked insect. Another drawback of infra-red illumination is that it probably precludes marking methods which depend on fluorescence, because this would occur at wavelengths further into the infra-red, beyond the spectral range (400 - 800 nm) of the video camera available for the study.

*The visible spectrum*

13. The reflectivity of vegetation to visible light (390 - 670 nm) is somewhat less than to near infra-red, but this gain is unlikely to outweigh the serious risk of modifying leafhopper nocturnal behaviour by using bright illumination in the visible spectrum.

*Near ultra-violet*

14. Near ultra-violet (300 - 390 nm) would be good for fluorescence marking methods, giving outputs in the middle of the spectral range of the camera. It would also be a good waveband to use because vegetation reflectivity tends to be low ($\approx$ 5%) in this region. A major disadvantage is that many insects are known to be strongly attracted to ultra-violet light. According to Abenes & Khan (1990), green leafhoppers are an exception to this rule, being more strongly attracted to yellow or green light, so they may perhaps not be unduly perturbed by ultra-violet illumination. On the other hand, other insects flying in the vicinity of the experimental area would almost certainly gather in large numbers near to the illuminator.

**Experiments**

15. Given the factors discussed above, it was decided to conduct a series of tests to establish the maximum field of view in which marked leafhopper-sized targets could be detected on video against a background of vegetation. The tests would be carried out using both near infra-red and near ultra-violet illumination, and with targets marked with reflecting and fluorescing materials, and with mixtures combining both characteristics.

*Video equipment*

16. A sensitive video camera (National Panasonic "Moonlight" series WV-1900), previously used for field studies of *Helicoverpa armigera* nocturnal flight (Riley *et al.*, 1990) was used in the tests. The camera was fitted with a zoom telephoto lens (f.1.6, 16 - 160 mm focal length), so that the field of view could be conveniently varied. The picture was displayed on a Phillips BM7502/05G 12-inch monochrome monitor.

*Reflecting materials*

17. If minute glass beads are partly immersed in a reflecting substrate, they tend to focus incoming illumination into spots at the bead-substrate interface. The light reflected from these bright spots is mainly returned on a reciprocal path through the beads, back towards the source of illumination. This concentration of returned light in the direction of the illuminating source (the "cat's eye" effect) greatly increases the reflectivity of the substrate in that direction compared to normal Lambertian reflection in which light is scattered into $2\pi$ steradians. The effect of high directional reflectivity is maintained over a range of incidence angles (typically up to 30° from the normal to the substrate). Reflectivity in the visual spectrum is optimised if the glass beads are approximately 60% immersed in a white or silver substrate (N. Parks, Potters-Ballotini Ltd., *pers. comm.*) We acquired a supply of glass beads of the type used to form high reflectivity coatings, in three size categories in the range 45 - 150 µm (Potters-Ballotini Ltd.), and these were used to make targets for assessment. Later, we also obtained some high-refractive index (RI) beads $\mu = 1.9$) in the size range 63 - 88 µm (Microbeads AG (UK) Ltd.).

18. Another form of commercially available highly reflective material is made from transparent plastic film, moulded into tiny pyramidal prisms, and sometimes bonded to a reflective backing. We obtained some microprism, retroreflective materials in a variety of colours, with and without silvering (Reflexite Ltd.). The thinnest of these materials were some self-adhesive tapes about 0.1 - 0.2 mm thick.

19. Reflecting targets were also made using aluminium foil, both alone, and as backing for the glass beads attached with various clear substrates.

*Fluorescing materials*

20. We had to hand samples of "Fiesta" daylight fluorescent dye powders (Haeffner & Co. Ltd.), of the type used in mark-recapture experiments, in three colours: "Stellar Green", "Solar Yellow" and "Astral Pink", with dominant emission wavelengths of 544.0, 569.5 and 495.5 nm respectively. To make targets for assessment, the powder was combined with Bostik Wood Adhesive Rapide, which dries to a clear and slightly flexible coating, and under test was found to have low toxicity to insects and reasonable strength (D. Winder, *pers. comm.*).

21. We also had available some "Correctine" fluid which fluoresces a bright pink/red. This liquid was at one time used by MOD typists as an amendment identifier on classified documents, but has now been withdrawn because of concerns about its carcinogenic properties.

*Illuminators*

22. Because our experiments were carried out in the Radar Unit dark room, we were limited to working at close range (~ 2 - 3 m). This, and the high sensitivity of the video camera, meant that only low power illumination was required. We therefore used a small, 3 V, "Mag-Lite" hand torch with a high-intensity krypton bulb (Mag Instruments Inc.), fitted with a narrow pass-band (2%) interference filter (Infrared Engineering Ltd), centred on 820 nm, as our near infra-red illuminator. When fitted with a 2% filter centred on 640 nm, the torch acted as a red light illuminator.

23. For our near ultra-violet illuminators we had two 9 inch mercury discharge fluorescent tubes of the type used in small light traps. One was a Phillips "Actinic 05" of nominal 6 W rating, with output primarily in the range 320-440 nm, and peak power of 0.19 W/nm at 370 nm. This produced a significant amount of light at the violet end of the visible spectrum. The other tube was a Sylvania F6T5/BLB "black light" tube, for which we had no specification data, but which was probably very similar to the Phillips tube, except that its output was confined to ≈ 320 - 390 nm, so that it was much less bright in the visible spectral region.

*Preliminary visual evaluation*

24. A preliminary assessment of the available reflective and fluorescent materials was made visually. Targets were produced by making spots of varying thicknesses (0.005 - 0.5 mm) on black card with a paintbrush, and these were examined in ambient lighting, then in low-level directional illumination in the darkroom, using visible (krypton-bulb hand torch) and UV ("black light" tube) sources. A selection of coloured Humbrol enamel paints, Bostik Wood Adhesive Rapide and Correctine were used alone and as substrates for the reflective beads.

25. Glass beads applied to the surface of a suitable substrate greatly enhanced the contrast of the targets in *directional* lighting, particularly at low angles of incidence. White and silver paint substrates performed best. Area density of the beads on the surface appeared to be an important factor, but mixing the beads with substrate eliminated the enhancement effect completely. The high RI beads performed better than the normal glass beads in all cases, with a single exception (on silver paint the 79 μm beads performed equally well). It was therefore decided to use only the high RI beads for experimentation.

26. The fluorescent dye powders and the Correctine gave good contrast enhancement in UV illumination, further improved by the addition of glass beads. The "Astral Pink" dye provided the best contrast against vegetation, so this and the Correctine were selected for experimentation.

27. The "Reflexite" (microprism, retroreflecting) tapes gave very good contrast enhancement. In visual light the white, grey and yellow tapes were brightest, but we considered that choice of illuminating wavelength would be very important, and so all the single colour materials thin enough to be attached to leafhoppers were tested.

*Experimental procedure*

28. The experimental arrangement is shown in Figure 1. In order to benefit from the directional reflectivity of the targets, the illuminators were placed adjacent to the camera. We found that the reverse side of X-band microwave absorber formed an excellent non-reflecting surface when used as a background for foliage (provided by a selection of potted plants) and targets.

29. Targets were produced by making 1.5 and 2.5 mm wide bands around 0.9 mm diameter glass capillary tubes, a similar diameter to leafhoppers. Details of the targets are given in Table I: they were mounted end-on in a putty base and viewed against the foliage. It was found that if the tubes were angled slightly from the vertical, away from the camera, unwanted specular reflection from the glass tubes was eliminated.

30. Groups of targets were placed together in front of the foliage, and the focal length of the lens was adjusted to maximum, when the targets could be clearly seen on the video monitor, against the vegetation. Then the brightness control of the monitor was steadily reduced, and the order in which the targets disappeared was noted. This procedure allowed us to (qualitatively) rank the targets in order of visibility against background. The procedure was repeated with the three different illumination sources. Examples of the monitor display with different levels of brightness setting are shown in Figure 2. The target with the best performance was added to the next group to allow cross referencing.

31. It is possible to increase the contrast of fluorescing markers by using a camera which is sensitive to only a narrow band of frequencies, centred on the marker emission band.
This procedure discriminates strongly against reflections from the background foliage of both illuminator and ambient wavelengths. We were able to conduct a preliminary assessment of this effect by using our near ultra-violet illuminators, and by fitting to the camera a narrow band filter centred on 640 nm. The camera was thus rendered insensitive to the illuminating wavelength, and could "see" only the red fluorescence from the markers.

32. Finally, the most visible target/illuminator combinations were selected, and the camera lens focal length was decreased (increasing the field of view) until the target was only just visible. The field of view at this point was noted. In order to prevent disturbance of the camera's automatic gain control being caused by reflections from the darkroom walls while target visibility was being assessed, a collimating tube was fitted to the lens.

*Supplementary tests*

33. We also investigated the practicalities of marking preserved *N.virescens*. A specimen was marked by attaching a rectangle of white Reflexite 0.8 x 0.3 mm lengthways along the top of the thorax using Bostik Rapide. The marked leafhopper and an unmarked control were mounted against the foliage (figure 3a) and examined under the most effective illuminators as established by the experiments above.

**Results**

34. The rank ordering of target visibility is shown in Table II. Amongst the first set of targets tested (ID nos. 1 to 6), white paint with microbeads (no. 5) performed best in all light wavelengths, and fluorescent dye powder/glue mix with microbeads (no. 3) performed next best, but separation between the two was very close in some types of illumination. Target 5 was therefore added to the second set of targets (nos. 10 to 16) as a comparison. In the second set of tests, the Reflexite targets (nos. 10 and 11) were brightest, usually by a significant amount, in all types of illumination. The colour which showed best performance varied with the type of illumination, but the white and grey Reflexite consistently performed well. Aluminium foil with microbeads in both clear nail varnish or Correctine (nos. 14 and 15) also considerably enhanced visibility, as did silver or white paint with microbeads (nos. 13 and 5). The relative performance of this group varied with illumination type.

35. Targets 7, 8 and 9 were examined briefly with no. 5, to evaluate the effect of decreasing marker size. As expected, the larger the marker, the greater the contrast enhancement and hence the field of view in which targets would be visible. However, it was clear that even very small numbers of microbeads adhering to a target can greatly enhance its contrast against foliage, in directional lighting.

36. The maximum field of view achieved with the most visible targets (those marked with white and grey Reflexite), was 1.7 $m^2$, when illuminated by the Actinite 05 UV tube (330 - 440 nm wavelength) (Table III). Good contrast and a field of view of 1.2 $m^2$ was achieved with red/near infrared illumination (640 nm).

37. In the experiment using UV illumination and a narrow band filter at 640 nm on the camera, the vegetation was quite invisible, and the fluorescent targets appeared as isolated spot images on the monitor. However, the images were bright enough to register on the camera only when the ultra-violet illuminator was placed about 0.5 m away from the target. This was probably because the narrow band filter was not matched to the pink/orange spectral output (possibly 610 - 650 nm) of the phosphor. Considerable improvement can be anticipated using a matched filter, and a more powerful illuminator, and this probability merits further investigation.

38. Visibility of the marked leafhopper was dramatically enhanced over the unmarked control and the foliage, as shown by figure 3b, over a fairly wide insect aspect range. The Bostik glue provided firm and satisfactory attachment, however, the Reflexite shape used would probably not be suitable for a live leafhopper, as it extended too near to the base of the wings. A triangular marker across the top of the thorax, widest at the junction with the head and tapering between the wings is expected to be the best compromise to maximise area without interfering with the leafhopper's behaviour.

## Conclusions

39. The experiments have shown that the visibility of leafhopper-sized targets against vegetation can be greatly enhanced in directional lighting by marking them with retroreflective materials of the type used in road signs and safety equipment. Amongst the marking materials tested, microprism, retroreflective tapes produced the greatest contrast against vegetation. Leafhopper-sized targets marked with this material, were visible over a field of view of 1.5 by 1.2 m. Glass microbeads on a suitable substrate were also successful.

40. The technique undoubtedly has considerable potential for observing the trivial (plant-to-plant) movements of small insects at night, but some further technical work is needed to optimise the UV fluorescence/narrow band filter method of observation. The next step in development would be to run a short laboratory trial using live insects, to ensure that the markers do not perturb their behaviour, followed by a field trial to test the marked insects in a realistic environment, recording their movements on video for analysis.

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**Table I: Details of experimental targets.**

| Target ID no. | Marker type | Marker materials | Remarks |
|---|---|---|---|
| **1** | Fluorescent | Glue dusted with fluorescent powder. | Some difficulty in getting powder to adhere. |
| **2** | Fluorescent | Glue mixed with fluorescent powder. | Easiest marker to apply. |
| **3** | Combination | Glue mixed with fluorescent powder and beads applied. | Difficulty in getting beads to adhere - small pressure had to be applied. |
| **4** | Control | Paint - Humbrol enamel matt white. | None. |
| **5** | Reflective | Paint (white) dusted with beads. | As 3. |
| **6** | Reflective | Aluminium foil attached with glue | Some difficulty in curving foil around tube without creasing. |
| **7** | Fluorescent | As no. 2, applied with grass blade to achieve finer band (approx. 0.5 mm) | Some difficulty experienced, as small amounts of substrate dried too quickly for beads to adhere. |
| **8** | Fluorescent | As above | As above. |
| **9** | Reflective | As no. 5, applied with grass blade to achieve finer band (approx. 0.5 mm) | As above. |
| **10a** | Reflective | White Reflexite | Easy to apply to tube, but adhesive probably not adequate for insects. |
| **10b** | Reflective | Blue Reflexite | As above. |
| **10c** | Reflective | Green Reflexite | As above. |
| **10d** | Reflective | Orange Reflexite | As above. |
| **11a** | Reflective | Grey Reflexite | As above. |
| **11b** | Reflective | Yellow Reflexite | As above. |
| **12** | Reflective | Paint - Humbrol enamel silver | None. |
| **13** | Reflective | Paint - Humbrol enamel silver dusted with beads | As 3. |
| **14** | Reflective | Aluminium foil coated with clear nail varnish and dusted with beads | Substrate tended to flake off when applied to tube. |
| **15** | Combination | Aluminium foil coated with Correctine and dusted with beads | As above. |
| **16a** | Control | Aluminium foil coated with clear nail varnish | As above. |
| **16b** | Control | Aluminium foil coated with Correctine | As above. |

**Table II: Target visibility results.**

| | Illumination | Target Visibility<br>Best ----------------------------->----------------------------Worst |
|---|---|---|
| **A** | UV visible | **5, 3, 6, 4, 2, 1** |
| | UV "black light" | **2, 3 & 5, 1, {4}, {6}** |
| | Hand torch + 490 nm filter | **5, 3, 4 & 6, {1 & 2}** |
| | Hand torch + 640 nm filter | **5 & 3, 4 & 6, 1, {2}** |
| | Hand torch + 820 nm filter | **5, 3, {4 & 6}, {1 & 2}** |
| | Hand torch + neutral density filter | **5, 3, {4}, {6}, {1 & 2}** |
| **B** | UV visible | **11a, 10ab, 5, 14, 15, 13, 12, 16** |
| | UV "black light" | **10b, 11a, 15, 13, 5 & 12, 14, {16}** |
| | Hand torch + 490 nm filter | **10ab & 11a, 14, 15, 5, 13, 12, {16}** |
| | Hand torch + 640 nm filter | **10ad, 11a, 13, 14, 15, 5, 12, {16}** |
| | Hand torch + 820 nm filter | **10abd & 11a, 14, 15, 13, 5, 12, {16}** |
| | Hand torch + neutral density filter | **11a, 10a, 14, 13 & 15, 5, {12}, {16}** |

1. Targets which did not show enhanced contrast relative to the foliage are listed in brackets.
2. Visibility of targets illuminated by the handtorch alone were severely compromised by specular reflection from the foliage and are not shown.

**Table III: Field of view of targets under different illuminating wavelengths.**

| Illumination | Width of field of view (m) | Area of field of view ($m^2$) | Remarks |
|---|---|---|---|
| UV visible | 1.50 | 1.73 | Good contrast with foliage. |
| UV "black light" | 0.495 | 0.19 | - |
| Hand torch + 490 nm filter | 0.80 | 0.49 | Good contrast with foliage. |
| Hand torch + 640 nm filter | 1.23 | 1.16 | Good contrast with foliage. |
| Hand torch + 820 nm filter | 1.125 | 0.97 | Poor contrast with foliage - limiting factor. |
| Hand torch + neutral density filter | 0.66 | 0.34 | Fair contrast with foliage. |
| Krypton bulb visible (hand torch) | 0.94 | 0.68 | Contrast compromised by specular reflection from leaves. |

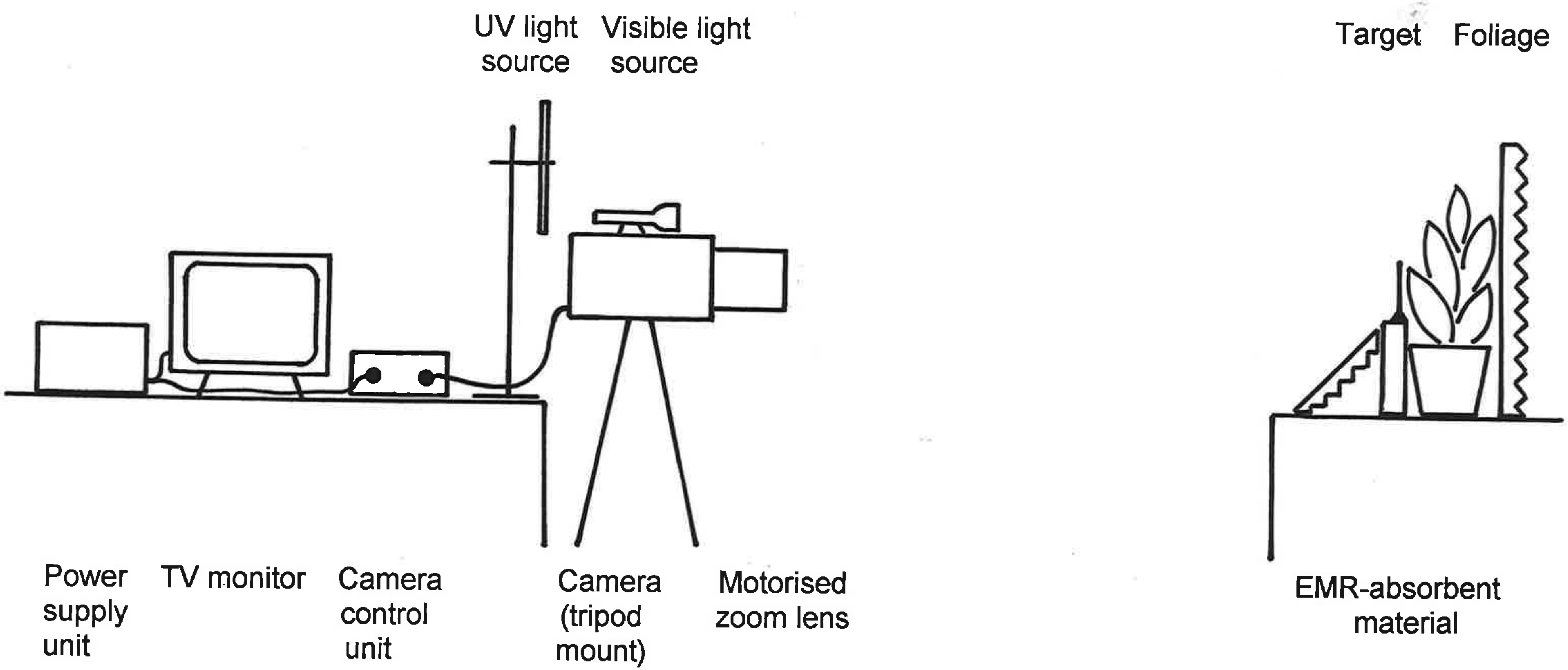


Figure 1: Schematic diagram of experimental set-up.

(a)

(b)

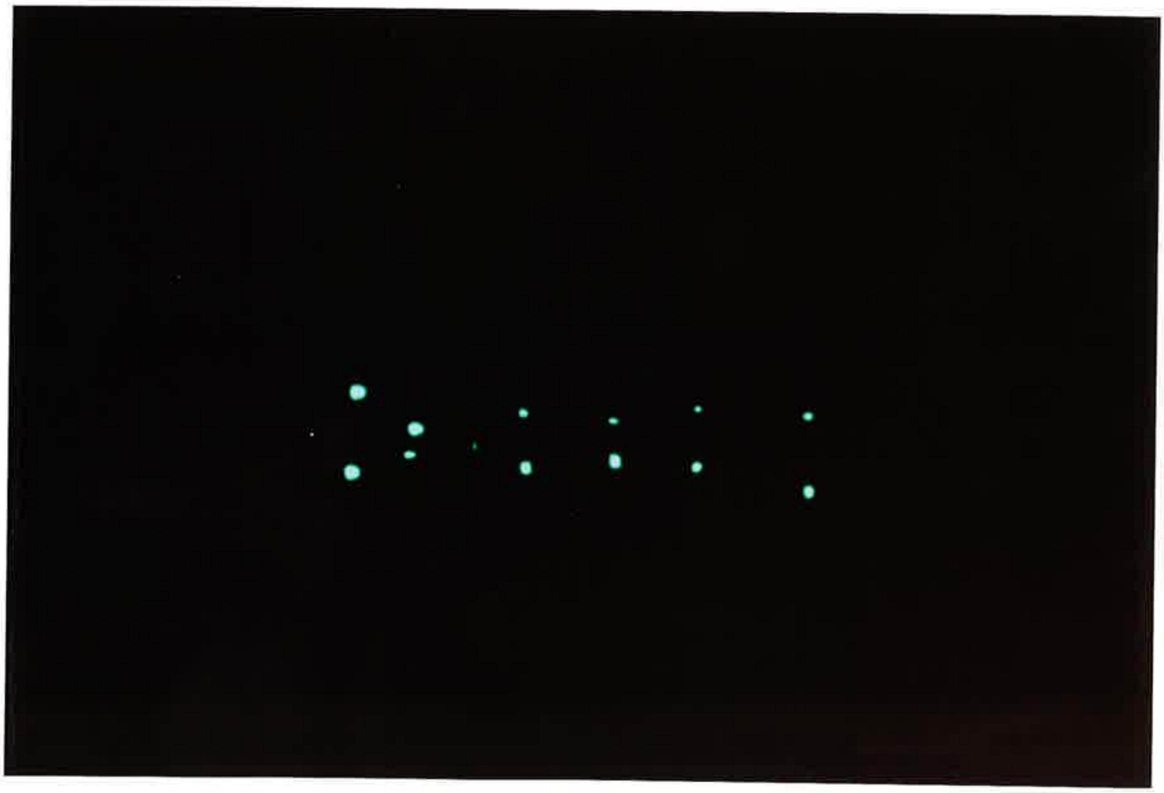

**Figure 2**: Examples of monitor display with different screen brightness settings. **(a)** The screen brightness is set high enough to show all the targets as very bright spots, but specular reflections from the leaves are also visible, complicating the picture. **(b)** At a lower setting the reflections from the leaves disappear, and as the brightness setting is progressively reduced, the less effective markers disappear one by one.

(a)

(b)

**Figure 3**: Example of visibility enhancement achieved by marking leafhoppers. **(a)** Close-up photograph in natural light of two *N.virescens* seen against foliage; the upper one carries a small Reflexite marker. **(b)** Video view from a range of 3 m: only the marked leafhopper is visible (bright spot) together with some specular reflections from the leaves.