A new approach for estimating egg parasitism of whitebacked planthopper

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Anagrus spp. are important egg parasitoids of whitebacked planthopper (WBPH) in ricefields in China. Egg parasitism, which ranges from 10 to 70%, is commonly estimated by dissecting rice tissue and recording numbers of healthy and parasitized host eggs. It is difficult, however, to detect parasitized eggs after wasps have emerged. Therefore, a simpler, more accurate method is needed.

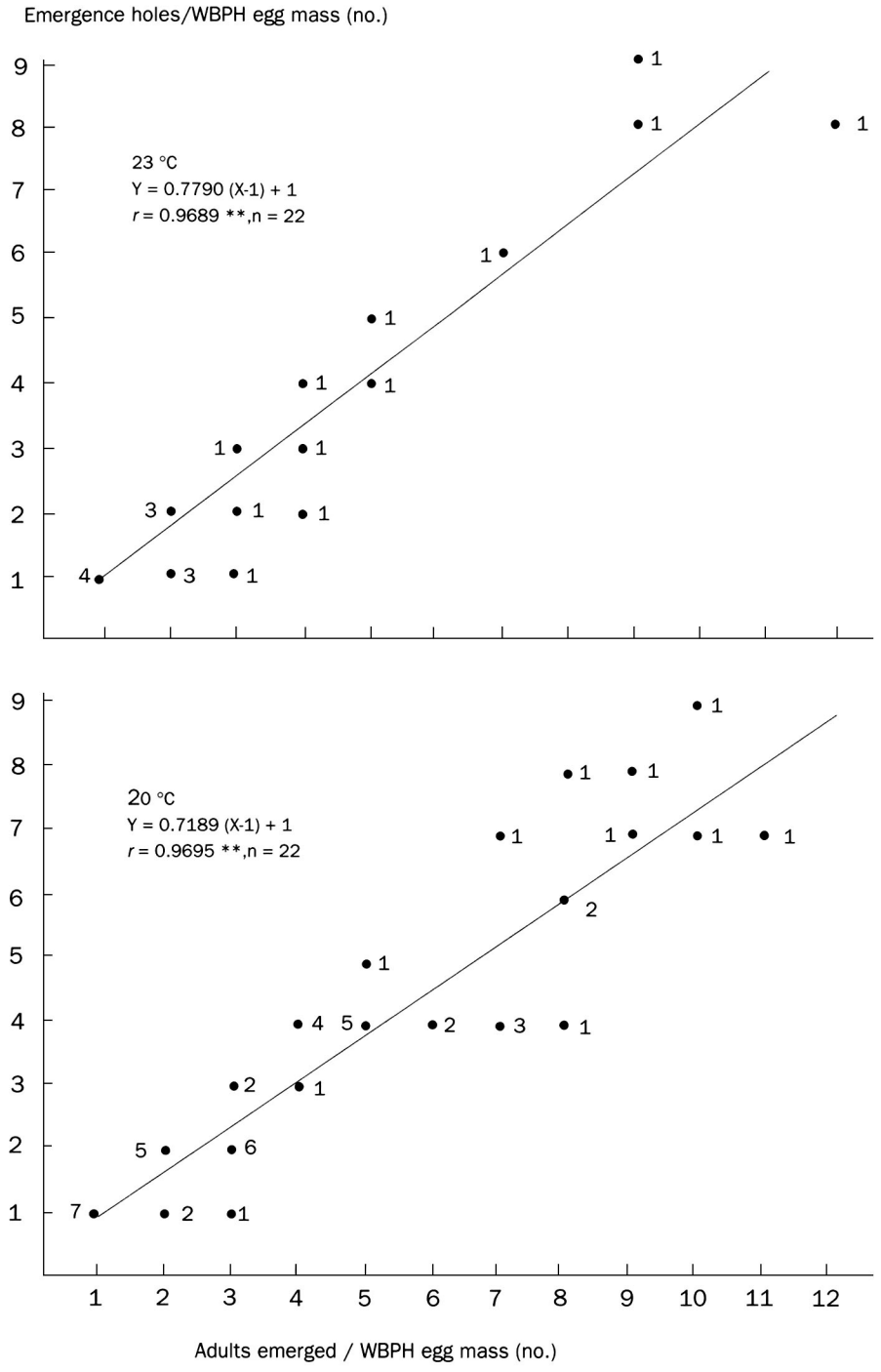
Previous studies showed that when *Anagrus* spp. have completed their development in WPBH eggs, they gnaw through the eggshells and rice plant tissues and escape through the resulting emergence holes. The relationship between number of emergence holes on tillering Guangluai 4 rice plants (about 5-10 mm in diam and 20 cm tall) and number of emerging adults of the egg parasitoid *Anagrus nilaparvata* Pang et Wang was measured in laboratory.

Female parasitoids were confined for 24 h in tubes enclosing hills of rice infested with WBPH eggs and then removed. Plants were kept in culture cabinets under suitable light (141: 10 d) and constant temperatures of 20° and 23° C. After offspring parasitoids emerged, the number of emergence holes on the rice plant surface and percentage of parasitism of WBPH eggs were determined using a stereomicroscope.

A linear regression function was obtained for each temperature (see figure). Slope values of *b* (0.7189, 0.7790) are less than 1, which indicates the parasitoids can emerge through emergence holes made by previously emerged parasitoids. Because the two slopes are not significantly different, the data sets from 20° and 23° C were pooled to obtain a common function:

$Y = 0.7267 (X-1)+ 1$, ($r = 0.9699$, $n = 71$, $P<0.01$)

Number of parasitized WBPH eggs can be estimated using the function



Relationship of number of emergence holes to number of emerged adults of *A. nilaparvata*/ egg mass of whitebacked planthopper (WBPH).[a] Figures near dots indicate number of observations at same values.

$X = 1.3761(Y-1)+ 1$.

Mean number of WBPH eggs/egg mass laid within tillering Guangluai 4 plants under different constant temperatures was measured in other independent experiments: 17 °C: 7.94 ± 0.68(17); 23 °C: 9.40 ± l.00(35); 26 °C: 7.93 ± 0.60(341); 30 °C: 6.04 ± 0.61(25). The parabolic relationship of number of WBPH eggs/egg mass against temperature is

$M = - 13.9722 + 2.1149T - 0.0484T^2$, ($r = 0.9764$, $P<0.01$).

A practical way to estimate egg parasitism is to collect WBPH-infested hills of rice on day t, and observe them in

the laboratory for about 1 wk for parasitoid emergence. Number of WBPH egg masses and parasitoid emergence holes can be counted under a stereomicroscope or magnifying glass. Parasitism then can be calculated as

$P(\%) = ([1.3761\ (A/n\text{-}1) + 1]/M) \times 100$

in which A = number of emergence holes, n = number of WBPH egg masses, M = mean number of WBPH eggs/egg mass under average daily mean temperature (T) from day t-10 to day t. ■

Applying rapid immunofilter paper assay to detect rice viruses

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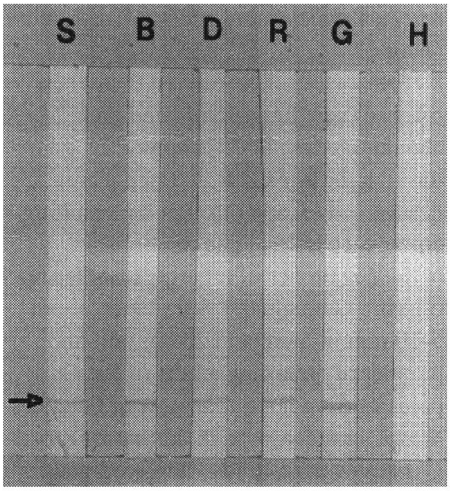
Rapid immunofilter paper assay (RIPA) was applied to detect nine rice viruses in extracts of plants infected with rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), rice grassy stunt virus (RGSV), rice stripe virus (RStV), rice gall dwarf virus (RGDV), rice black-streaked dwarf virus (RBSDV), rice ragged stunt virus (RRSV), rice dwarf virus (RDV), or rice transitory yellowing virus (RTYV).

Two kinds of latex beads (Japan Synthetic Rubber Co. Ltd., Tsukuba) were used in RIPA: white latex as the solid phase and pink latex as the tracer. The white and pink latices were separately sensitized with specific antiserum. A thin coat of sensitized white latex was applied on Whatman glass filter paper strips (Whatman GF/A; 0.5 × 9 cm) about 1.5 cm from the lower end. Latex-coated filter paper strips were stored in a desiccator at room temperature until use, while the sensitized pink latex suspension was kept at 4 °C.

For virus assay, 0.1 g each of healthy and infected rice leaves were separately ground in 900 µl of extraction buffer (TBS = 0.02 M Tris-HCl, 0.15 M NaCl, pH 7.2 with 0.01 M Na_2SO_3) using mortar and pestle. The extracts were clarified by centrifugation at 15,000 rpm for 10 min. Clarified sap was serially diluted twofold with extraction buffer. One hundred microliters of clarified extract was placed in flat-bottomed Eppendorf tubes in which the lower end of the latex-coated filter paper strip was dipped until the extract was fully absorbed. Then, 100 µl of IgG-coated pink latex (diluted to 0.025% [vol/vol] with TBS) was added to the same tube. The pink latex suspension moved upward by capillary action and a pink band appeared on the spot where the white latex was applied, indicating a positive reaction (see figure).

RIPA efficiently detected the virus antigen at different dilutions on infected sap but not on healthy sap (see table). The sap dilution end points depended on concentration of the virus in the plant and the titer of the antiserum. For RStV and RGSV, which have high reactivity to their antibodies, a negative reaction was observed at low dilutions of the extract from infected plants. A higher dilution (640-1280 x) is, therefore, recommended for their detection in RIPA. Optimum sap dilutions for virus detection were determined for each virus. Dilution range that gave the brightest pink band in at least three trials was considered optimum for a given virus. Optimum sap dilutions for other viruses were 40-80 x for RTBV and RTSV, 640 x for RDV and RTYV, and 80-160 x for RGDV, RRSV, and RBSDV. The sensitivity of RIPA is comparable with enzyme-linked immunosorbent assay (ELISA) when tested on RTBV, RTSV, RGSV, and RDV.

RIPA is simpler, less time-consuming, and less expensive than ELISA and does not require sophisticated equipment. RIPA is as simple as using pH test paper strips. Coated filter paper strips can be prepared in advance and stored in a desiccator for about 6 mo. ■



Detection of rice viruses by RIPA: S = RTSV, B = RTBV, D = RDV, R = RPSV, G = RGSV, H = healthy check. Visible bands (arrow) indicate a positive reaction.

Detection of RTBV, RTSV, RGDV, RDV, RStV, RGSV, RRSV, RTYV, and RBSDV in rapid immunofilter paper assay.[a]

Virus	Reciprocal of dilution										
	10	20	40	80	160	320	640	1280	2560	5020	10240
RTBV	+	+	+	+	+	+	+	+	–	–	–
RTSV	+	+	+	+	+	+	+	–	–	–	–
RGDV	W	W	+	+	+	+	+	+	+	+	–
RDV	+	+	+	+	+	+	+	+	+	+	–
RStV	–	–	–	W	+	+	+	+	+	+	–
RGSV	–	–	–	–	W	+	+	+	+	+	–
RRSV	+	+	+	+	+	+	+	–	–	–	–
RTYV	+	+	+	+	+	+	+	+	+	–	–
RBSDV	+	+	+	+	+	+	+	+	+	–	–
Healthy sap	–	–	–	–	–	–	–	–	–	–	–

[a] + = strong positive reaction, – = negative reaction. W = weak reaction.