Selected Aquatic Insects Can Reduce Stands in Water-Seeded Rice

Previous research at the Texas AgriLife Research and Extension Center at Beaumont on water-seeded rice revealed the possibility that one or more unknown aquatic invertebrate species have been responsible for dramatically reducing rice stands. Preliminary experiments show that there is zero or small reductions in the rice stand of plots when treated with insecticide applied to rice seed, applied to the soil before flooding, or applied to the water surface before emergence of rice seedlings through the water surface. In contrast, plots that did not receive insecticides, or received insecticides relatively late, exhibited significant stand losses. These results suggest that a biological organism impacted water-seeded rice during the early seedling stages and is responsible for stand reductions.

Field and greenhouse experiments were conducted at the Texas AgriLife Research and Extension Center to identify the causes of these rice stand losses. In the field, metal barriers were installed around plots, which were flooded and water-seeded (continuous or pinpoint flood). These barriers were pushed down in the mud and served to isolate plots. Some plots were treated with insecticide at various times during seedling development, while other plots were left untreated. Plots were sampled over time for benthic and aquatic invertebrates, and for stand losses. Dislodged seedlings were also counted and removed from plots at various times after seeding (Fig. 1). Organisms sampled were preserved and identified. Plots were harvested and yields compared. During these experiments, a common aquatic beetle, *Tropisternus lateralis*, caused dislodging of plants and their floating to the surface of the water. This beetle does not actually feed on rice, but feeds on detritus, which may be associated with rice seedlings. We also discovered that another aquatic beetle, *Berosus infuscatus*, was abundant and was a possible candidate responsible for the stand losses. Both of these beetles were negatively impacted by insecticide treatments.

Results of field experiments showed that dislodging of plants occurred when *T. lateralis* was present and active during a narrow time period of about 10 to 14 days after rice germination, but before seedling emergence through the water surface. Also, insecticides applied after rice emergence through water had little effect on dislodging. In addition, more dislodging occurred in the continuous irrigation system than in the pinpoint flood irrigation. Most dislodging occurred in untreated plots or in plots treated with insecticide relatively late. In general, stand counts and grain yields were lower in untreated plots, but rice displayed an ability to compensate for...
Welcome to the June issue of Texas Rice. I hope you have the opportunity to attend either the Eagle Lake or Beaumont Field Day. The theme for both field days is “Harvesting the Future”.

The 34th annual Eagle Lake Field Day begins at 4 PM on Tuesday, June 24, at the Texas AgriLife Research David R. Wintermann Rice Station in Eagle Lake. The field tour will include a visit to the station’s research plots with five presentations given by our scientists. Garry McCauley will kick off the tour by discussing his research on the performance of hybrid and conventional inbred varieties. This will be followed by a second presentation by Garry on phosphorus and potassium application rates. Dante Tabien will then describe research on the high-yielding, water-efficient rice plant type being developed in conjunction with the Lower Colorado River Authority and the San Antonio Water System, which will be followed by a presentation by Anna McClung on conventional rice varietal development. Mo Way’s presentation on seed treatment insecticides and insect management will wrap-up the field tour. The evening program will include a presentation by L. G. Raun, who will talk about the farm bill. John Miller with Southwest Agribusiness Consulting Inc. will follow with a rice industry outlook update. The evening program will be followed by a barbecue dinner.

The 61st annual Beaumont Field Day begins at 8 AM on Thursday, July 10, at the Texas AgriLife Research and Extension Center in Beaumont. The field tour will include presentations by Dante Tabien on rice varietal development, Shannon Pinson on rice genetics and molecular biology, Ted Wilson on rice inbred/hybrid photosynthesis and physiology, Lee Tarpley on soils and plant nutrition, Abdul Mohammed on plant physiology, Mo Way on rice insect management, and Young-Ki Jo on rice plant pathology. The field tour will be followed by a morning program, which will begin at 10:45 AM. Ted Wilson, the Beaumont Center Director, will give an overview of the Center’s research activities, Texas Department of Agriculture Commissioner Todd Staples will provide an overview of TDA’s role in promoting Texas Agriculture, and Michael Salassi, who is the Louisiana State University J. Nelson Fairbanks Endowed Professor of agricultural economics, will discuss commodity pricing and economics. The morning program will be followed by a luncheon. At about 1 PM, our afternoon tour will begin, with a presentation by Lee Tarpley, Mo Way, and Ted Wilson on the Center’s bioenergy and alternative crop production research.

As the previous paragraphs indicate, this year, we are hosting our 34th field day at Eagle Lake and our 61st field day at Beaumont. We anticipate 175 to 250 visitors at the Eagle Lake Field Day and 325 to 450 at the Beaumont Field Day. These numbers far exceed what is typically observed at most field days at research and extension centers in other areas of Texas. The high attendance and participation at our Eagle Lake and Beaumont Field Days is largely the result of the strong support provided by our agricultural industry, from growers, to seed producers, to equipment, fertilizer, to pesticide manufacturers. Every year, the rice industry donates funds, equipment, and door prizes for our field days. Last year’s donations topped $16,000, with BU Growers leading the list of over 100 companies and individuals who provide donations by covering the cost of the Eagle Lake barbecue and

Continued on page 8
The Interaction Between Temperature and Planting Date and Their Impact on Rice Grain Quality and Yield

Temperature and Planting Date in Texas

Temperature is one of the major factors that impact a farmer’s ability to produce a successful rice crop in Texas. By planting in early March, the main rice crop completes its maturity prior to the hottest summer temperatures that have the greatest negative impact on heading and grain filling, while allowing the ratoon crop to mature sufficiently early to avoid the cold autumn temperatures. Planting still earlier, while having an advantage for the ratoon crop, places the crop at risk of cold fronts that can cause major damage to the rice crop and require replanting. In general, temperatures cause stress to rice crops when they are below 65°C or above 95°C.

For most cultivars, the optimum night temperature is generally from 77 to 84°F, which is about 3.6 to 7.2°F lower than the optimum day temperature [3]. Furthermore, the effects of day temperature and night temperature on developmental rate depend on the type of rice that is being produced. For example, japonica cultivars show a greater difference in their developmental rate response to changes in day or night temperature than indica cultivars.

Historically, predicting the impact of daytime and nighttime temperatures on rice development has been difficult due to limited availability of easily accessible climatic data, and the lack of computer tools that can be used to predict the impact of temperature on crop growth and development. A major emphasis of the Texas AgriLife Research and Extension Center at Beaumont has been to address both of these deficiencies. The Integrated Agricultural Information Management System [1] was developed to provide near real-time climatic data for each of the nearly 70 rice-producing countries of the world. Data for over 20,000 climatic stations can be accessed at http://beaumont.tamu.edu/ClimaticData/ in an easy to use format. For Texas rice producers and researchers, the Rice Development Advisory (RiceDevA) [2], can be accessed at http://beaumont.tamu.edu/RiceDevA/RiceDevA.aspx and used to predict the impact of temperature on a wide range of historic and current

Fig. 1. Seasonal dynamics of daily maximum and minimum temperatures at Beaumont, based on historic data available through the Integrated Agricultural Information System at http://beaumont.tamu.edu/ClimaticData/.
Temperature and Rice continued ...

rice cultivars grown in Texas. RiceDevA can be used as a tool in scheduling planting so that the growing period of a specific rice cultivar falls within historically favorable weather. For example, using data for Beaumont, the seasonal trend in temperature shows the window of desirable and undesirable temperatures for rice crop production at Beaumont (Fig. 1). And, using one of the features of RiceDevA, estimates of the dates of crop stages (2nd tiller stage, panicle differentiation, heading, milking stage, and maturity) of a specific cultivar (e.g. Cocodrie) can be obtained for different planting dates (e.g. planting is done on February 15 and every 7 days after until May 30) (Fig. 2).

In the Texas Rice Production Guidelines [4], Dr. Lee Tarpley and his colleagues state that the optimum planting dates range from March 15 to 21 for the western rice planting area and from March 21 to April 21 in the eastern area. However, in Texas rice farms, planting dates generally range from the last week of February to the last week of May. In neighboring Crowley, LA, Dr. Nathan Slaton and colleagues [5] estimate the yield loss percentage at various planting dates. They estimate that yield loss at Crowley would be 0% if planting date was March 8, 5% at April 9, 10% at April 22, 15% at May 2, 20% at May 10, 25% at May 18, and 30% at May 24.

**Low Temperature Effects on Rice**

Although planting early is done to avoid the hot temperatures that negatively affect the main crop, there is obviously greater risk of low temperature negatively impacting crop development. When rice seedlings experience cold weather, the new leaves that emerge lack pigments, due to cool temperature-induced chlorosis. The damaged leaves are white and do not recover even when favorable temperatures return [6].

Low temperatures also slow the rate of crop growth and increase the number of days to maturity. For example, the average number of days to maturity of the main crop was 5 days longer in an early-planted (March 9, 2007) preliminary yield trial (PYT) compared to that in a regular PYT (March 22, 2007).

There is also concern over the negative effect of low temperatures on the ratoon crop, especially with late maturing cultivars that are planted late (around May). It is possible that there may be a few days of cold weather and this may affect yield, especially if the cold weather occurs during the floral to booting stages.

Summarized below are the effects of cold temperature (59°F day and 50°F night) on several rice traits of cold-tolerant and cold-sensitive cultivars based on research by Jacobs and Pearson [7]:

- **Spikelet Numbers.** The plant stage when cold temperatures occur affects the amount of the reduction in number of spikelets on main culm panicles. Cold temperatures that occur at floral initiation and booting reduce main culm spikelet numbers by 41% and 15%, respectively. The duration of cold temperatures also affect main culm spikelet numbers, which remain high at 91% if the cold temperatures last for 2 days and

![Fig. 2. Output obtained from the Rice Developmental Advisory (http://beaumont.tamu.edu/RiceDevA/RiceDevA.aspx) showing the growth stages of Cocodrie if planted weekly from February 15 to May 30 based on historic data of Beaumont, TX.](image-url)
decreases to 58% if cold temperatures last for 5 days.

- **Spikelet Fertility.** When cold temperatures occur at booting, the number of spikelets and spikelet fertility are affected. Five days of cold temperatures reduce these traits by 16 and 59%, respectively.

- **Apex Growth.** Apex elongation (length of the main stem apex) stops during cold temperatures, but resumes when temperatures increase in both cold-tolerant and cold-sensitive cultivars, resulting in apex lengths that are smaller than that of the non-cold treated plants (controls). Apex lengths of cold-treated plants (5 days of cold), relative to the non-treated controls, are about 55 and 35% for the cold-sensitive and cold-tolerant cultivars, respectively.

- **Panicle Branches and Lengths.** The number of panicle branches and panicle length is lower by 43% and 30%, respectively, when exposed to 2 days of cold temperatures.

- **Photosynthesis.** Leaf-level carbon dioxide exchange rate (CER) increases as temperature increases from 45.5°F to 77.0°F for cold-tolerant cultivars, and from 45.5°F to 81.5°F for cold sensitive cultivars. Furthermore, CER declines rapidly as temperature exceeds 86.0°F.

**Cold Tolerance and its Screening at Texas AgriLife Research**

Rice is cultivated across a wide range of environments worldwide and its different subspecies have different reactions to low temperature. Temperate *japonica* cultivars have better seedling-stage cold tolerance (they are less chlorotic at 48.2 and 55.4°C) and higher seedling vigor than *indica* cultivars [8]. Rice breeders select lines that are adapted to their growing environments, and for temperate regions or high altitude locations in the tropics, cold tolerance is one of their goals. Quantitative trait loci that confer cold tolerance during the seedling stage and the booting stage have been identified in rice.

The rice breeding team at the Texas AgriLife Research and Extension Center at Beaumont, headed by Dr. Rodarte Tabien, screens seedlings from its breeding lines for cold tolerance. Seedling cold tolerance is critical if earlier planting dates are to become more common to avoid high temperatures later in the season. The screening procedure that they apply includes sowing test entries in flats and exposing them to the cold weather of February. Air and soil temperatures are monitored throughout the 29-day duration of the experiment and each line's cold tolerance rating (1 = best to 5 = poor) and seed germination percentage is estimated. Desirable lines are those that score high in both categories.

Figures 3 and 4 show the response of the entries to a February test that had five days of freezing temperatures. Average temperatures during the test period included: maximum and minimum soil temperatures of 61 and 52°F, respectively; and maximum and minimum air temperature of 67 and 46°F, respectively. Several genotypes performed well, relative to Cocodrie, and these are being used in
Temperature and Rice continued ...

In contrast to the cold temperatures that occur during the start and end of a rice cropping season, high temperatures for most late-planted rice fields can cause low yields. The negative effects of high temperatures on the rice crop have been summarized below:

**Carbohydrates.** Total non-structural carbohydrates (TNC), which are the sugars, starches, and fructosans, can be accumulated and translocated for metabolism. In rice, TNC is accumulated in stems until heading and can be a major contributor to grain TNC during grain filling. In 1995, it was observed that TNC concentration in rice stems decreased as a result of five consecutive days of maximum daily temperatures that exceed 95°F, and it was suggested that the high temperatures increased maintenance respiration, causing an increased loss in TNC [9]. This decreasing TNC trend at high temperatures was observed in 12 of the 15 rice genotypes evaluated, and interestingly, the two earliest maturing genotypes did not show this trend.

**Filled Grain Percentage.** High temperatures during flowering in rice decreases the ability of the pollen grains to swell resulting in poor dehiscence, while high temperatures just a few days before flowering lowers the function of the thecae (part of the anther that contains the pollen) to dehisce, causing even poorer dehiscence [10]. Sterility is induced by high temperature (95°F) prior to heading, peaking around nine days prior to heading corresponding to the young microspore stage [11]. As a result, high temperature induces floret sterility and low filled grain percentage. In 2006, the decreasing daily minimum relative humidity and increasing in maximum daily temperature from July 10 to 22 coincided with the heading dates of entries in a yield trial planted on April 14 at the Texas AgriLife Research and Extension Center at Beaumont. When

Fig. 4. Cold tolerance phenotypic score of selected rice germplasm and Cocodrie (CCDR) evaluated at field temperature at Beaumont in February 2006.

Fig. 5. Selected germplasm with seedling cold tolerance planted in February 2008. Note the two yellowing and short susceptible entries at the right side. (Photo by Patrick Frank)

developing populations. Recent screening identified new germplasm and mutation-derived lines that have seedling cold tolerance (Fig. 5).

**High Temperature and Rice**

In contrast to the cold temperatures that occur during the start and end of a rice cropping season, high temperatures for most late-planted rice fields can
Temperature and Rice continued ...

filled grain percentages were determined, Cocodrie (the early maturing check cultivar, which headed on July 10) had 82% filled grains, while Wells (the late-maturing check, which headed on July 20) had 58% low filled grain. Yield trial entries that headed later than July 10 had lower filled grain percentages than Cocodrie. In a separate study that was also planted on April 12, Banks headed on July 24 and produced only 52% filled grain. As a result, yield trials were planted earlier in the succeeding years to avoid the unfavorable effect of high temperatures and low relative humidity on later maturing yield entries.

Grain Quality. The panicle is the most sensitive organ to high temperature and the fluctuation in grain quality of rice is usually partly attributed to varying temperatures during kernel development. In several studies conducted in Japan, high temperature decreased grain filling, kernel mass and grain size, and increased grain chalkiness. Even the classification of rice based on amylose content can be changed by growth temperature. Low temperature decreases amylose content of high amylose cultivars and increases amylose content of low amylose cultivars.

High night temperatures decrease grain size, which in turn affects head rice yield. It is speculated that high night temperature is important in head rice yield. A study by Cooper et al. [12] utilized the staging of rice proposed by Counce et al. [13] and 17 years of weather data and head rice (HR) yield to study the relationship of low and high temperature and head rice yield of Lemont and Newbonnet. Their results indicate that high day temperature at R6 (beginning of grain filling) reduces HR yield, but high day temperature at R7 and R8 (when one kernel of the main panicle turns brown) increases HR yield. Overall, the high night temperature at R8 was the most influential factor on HR yield. Since the R8 growth period spans about 12 to 14 days and occurs between mid-August and mid-September in Southern U.S., it was suggested that early planting or the use of early-maturing cultivars are the best options to avoid the high night temperatures that occur at grain filling stage. For better HR yield, when possible, fields should be planted to allow a greater percent of grain filling to occur during June, which is a cooler month compared to July or August. A subsequent study by the same authors using growth chambers and varying night temperatures (64.4, 71.6, 78.8, and 86.0°F) from 12 am to 5 am, starting at the R5 stage (one kernel of the main panicle starting to fill with starch), demonstrated that high night temperature affects HR yield. Increasing night temperature decreases HR yields in two hybrids (XP710 and XL8), M-204, and LaGrue, but not in Cypress (a consistent good miller) and Bengal. The temperatures tested caused significant changes in the chemical properties of the kernel, the maximum amount of force to break kernels of brown rice, and grain size, but the most profound change was in the chalkiness of the grain. High temperature favors the down-regulation (decrease synthesis or production) of genes related to starch synthesis and the up-regulation (increase synthesis or production) of genes related to starch-consuming α-amylases and heat shock proteins. Grain that developed at high temperature had low amylose content and a high amount of long chain-enriched amylopectin, but these were not related to increased chalky grains at high temperatures [14].

For more information, please consult the following references:


* Article by Drs. Stanley Omar PB. Samonte, Lloyd T. Wilson, Rodante E. Tabien, and Yubin Yang, Texas AgriLife Research and Extension Center, Texas A&M University System, Beaumont, TX.

Beaumont luncheon. Without this strong support our field days would be much smaller and would be more in line with what is hosted at the 12 Centers located in other parts of our state. The cost of the meals alone will exceed a third of this amount, with field signs and posters, and a large number of other expenses taking large chunks as well. We are very appreciative of this support and hope we can make the field days informative events for everyone who attends.

Monetary support for our field days has begun a subtle change this year. Historically, all of our support has been directly related to the rice industry. This year, we began to receive funding support for the Beaumont Field Day from the energy/petroleum industry sector. This change is a reflection of recent national and regional emphasis on cellulosic bioenergy production and the corresponding effort by our scientists to obtain grant support and conduct research on bioenergy crops breeding, production, and management. The diversification that a bioenergy crop industry has the potential to bring to the Texas Upper Gulf Coast would be very welcome. I anticipate bioenergy research will play an increasing role at our Center.

I would be remiss were I not to mention the tremendous monetary support provided by the Texas Rice Research Foundation through the Texas Rice Producers Board. The research we do at Eagle Lake and Beaumont, is all about our growers. Each and every year, these organizations fund from $400,000 to $800,000 in rice research. The rice growers who these organizations represent, through their support, shape the research that we do and are the main reason we are here.

Please keep on sending me your suggestions.

Sincerely,

L.T. Wilson
Professor and Center Director
Jack B. Wendt Endowed Chair in Rice Research
stand loss.

In the greenhouse, aerated covered bins were water-seeded and infested with varying densities of *T. lateralis* and *B. infuscatus* (Fig. 2). Dislodged plants were counted and removed, and the insects were observed for mortality (dead insects were replaced with live ones) and plant feeding behavior. Results of the greenhouse experiments were dramatic (Fig. 3). *T. lateralis* caused dislodging, while *B. infuscatus* did not. The higher the density of *T. lateralis*, the higher the number of dislodged plants.

Fig. 2. Greenhouse experiment for the identification of organisms responsible for dislodged rice plants. (Photo by Becky Pearson)

In conclusion, the results strongly suggest that *T. lateralis* is responsible for dislodging rice seedlings in the water-seeded regime. Foraging activity appears to be associated with this damage. Observations also suggest that the female *T. lateralis* may be more responsible for dislodging seedlings than males because of egg laying activities. More research is needed to confirm these observations. Pinpoint flooding appears to be a potential cultural method of preventing stand loss due to aquatic beetles. Increasing seeding rate is another cultural control tactic. Insecticidal seed treatments and early applications of pyrethroids before rice emergence through water offer an alternative control option. Water seeding is no longer the normal practice in planting rice in Texas, but most organically-produced rice is water-seeded. In addition, water-seeding is still common in southwest Louisiana and California, while more rice acreage is being water-seeded in Arkansas and Missouri. Aquatic beetles are common in these rice-producing areas. It should also be noted that these beetles are not the sole cause of plant dislodging in water-seeded rice. Wind also plays an important factor and can work in concert with biotic agents in causing excessive stand losses.

Fig. 3. Effect of *Tropisternus lateralis* density on the number of dislodged seedlings (A) and plant stand (B).

in southwest Louisiana and California, while more rice acreage is being water-seeded in Arkansas and Missouri. Aquatic beetles are common in these rice-producing areas. It should also be noted that these beetles are not the sole cause of plant dislodging in water-seeded rice. Wind also plays an important factor and can work in concert with biotic agents in causing excessive stand losses.*

*Article by Becky Pearson, Dr. Mo Way, Mark Nunez, and Luis Espino. Becky, Mo Way and Mark Nunez are with the Texas AgriLife Research and Extension Center, Texas A&M University System, Beaumont, TX. Luis Espino is a former Ph.D. student of Texas A&M University, who conducted his Ph.D. research at Texas AgriLife Research.
The Texas rice-breeding program focuses a significant amount of its effort developing shorter stature rice cultivars. Plants that are too tall tend to lodge, which reduces grain yield. Since 1981, shortened plant height has been achieved in almost all Texas cultivars by incorporated the semi-dwarf (sd-1) gene, which shortens the length of the stem internodes and increases the harvest index (ratio of grain mass to plant mass). Both of these factors have contributed to increasing the yield potential of semi-dwarf cultivars. The semi-dwarf (sd-1) phenotype results from a deficiency of active gibberellins (GAs) in the elongating stem arising from a defective 20-oxidase GA biosynthetic enzyme [1]. Although most current cultivars possess the sd-1 gene, there are still some cultivars that are released without it. Currently, semi-dwarf cultivar plant heights are around 37.4 inches (mean of Saber, Hidalgo, Presidio, and Sabine in the Uniform Regional Rice Nursery [URRN] across 2005 and 2006).

Plant height is commonly measured in the URRN, which is a multi-environment (year and location) trial (MET). Plant height is significantly affected by genotype, environment, genotype x environment interaction (GEI), and nitrogen. Significant GEI implies that there are some genotypes that are stable and some that are unstable in terms of plant heights when grown across different environments. A useful tool that can be used to analyze and rank the performance and stability of cultivars, in terms of plant height, is the genotype plus genotype x environment interaction (GGE) biplot [2]. The GGE biplot can be used to identify which rice cultivars have stable plant heights and which have unstable plant height across environments, and also determine whether cultivars with the sd-1 gene are more stable than those without it. This would provide an insight as to the expressivity of the sd-1 gene in different genetic backgrounds.

Plant height data for 18 cultivars grown in 5...
locations for each of 3 years (2005 to 2007) as part of the URRN were used in this study. Thirteen of these cultivars possess the \textit{sd-1} gene (Bengal, Cheniere, Cocodrie, Cybonnet, Cypress, Dellrose, Dixiebelle, Hidalgo, L205, Presidio, Priscilla, Sabine, and Trenasse), while 5 do not (Banks, Francis, Jupiter, Spring, and Wells).

Analysis of variance was used to determine the effects of cultivar, location, year and their interactions on plant height. Cultivar, location, year x location, and year, were all significant, explaining 36.7, 19.9, 16.4, and 2.7% of the variation, respectively. Mean plant height was significantly lower in the cultivar group that had the \textit{sd-1} gene (37.4 inches) than in the group without this gene (40.6 inches) (Fig. 1). Four (Banks, Spring, Wells, and Francis) of the five cultivars that do not have the \textit{sd-1} gene were taller than the overall mean plant height of 38.2 inches. However, Jupiter, which also does not have the \textit{sd-1} gene, was the third shortest at 37.0 inches.

Greater variation in plant height was found in the cultivar group that had the \textit{sd-1} gene in LA and in MO (when averaged across locations), and when averaged across all environments (year-location combinations) (Fig. 2). Further analysis show that Wells, Banks, and Francis were the most stable cultivars for plant height, while Cheniere, Priscilla, and Spring were the least stable (Fig. 3). Cybonnet, Cocodrie, L205, Dixiebelle, and Bengal had a less stable plant height than Jupiter. The variation in plant height suggested that stability in the expression of the \textit{sd-1} gene differs depending on the genetic background in which the gene is incorporated.

A study in 2002 showed different transcription levels of \textit{sd-1} in three cultivars (IR24, Milyang 23, and Habataki) [3].

References for this study are listed below:


* Article by Dr. Stanley Omar PB. Samonte and Dr. Rodante E. Tabien, Texas AgriLife Research and Extension Center, Texas A&M University System, Beaumont, TX.
Rice Crop Update

As of June 13, 2008, 100% of the Texas rice acreage had been planted, 100% had emerged seedlings, 90% had reached the permanent flood stage, 47% had reached the panicle differentiation stage, and 3% had reached the heading stage.