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Field Crops Research

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https://doi.org/10.1016/j.fcr.2024.109304

Field Crops Research 309 (2024) 109304

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Cotton crop transpiration reveals opportunities to reduce yield loss when applying defoliants for efficient mechanical harvesting

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ARTICLE INFO

Keywords:
Boll opening percentage
Defoliation strategy
Heat ratio method (HRM)
Machine-harvested cotton
Sap velocity

ABSTRACT

Chemical defoliation is an essential pre-harvest practice in machine-harvested cotton production in China, in which the timing of spraying chemical defoliants (harvest aids) is the key to ensure the yield and quality without loss due to weather risks at harvest time. This study aimed to find out based on crop transpiration if chemical defoliants could be sprayed early without yield loss. Field experiments were carried out in 2020 and 2021 in Xinjiang, China. The treatments consisted of a defoliant treatment with harvest aids (a mixture of 10% thidiazuron and 40% ethephon) sprayed in mid-September and water as a control, under four typical sowing patterns. Sap flow was measured during the defoliation period using a Heat Ratio Method (HRM) and then calculated as transpiration per unit ground area and per unit leaf area. The boll opening percentage in the defoliation treatment (84.9%) was higher than the control (77.3%), resulting in a 14.8% higher cotton lint yield. Row spacing and plant density did not affect lint yield and the defoliation percentage. Daily transpiration per unit ground area under the defoliants treatment was 1.26 mm d^{-1} and 39.1% lower than in the control (2.07 mm d^{-1}), and the daily transpiration per unit leaf area was 11% lower than the control. The daily transpiration per unit ground area did not change significantly in the first four to five days after spraying, and then started to decrease rapidly. This indicated that the crop could continue to grow and develop the first days after applying defoliants, thereby reducing yield loss. The results of this study show it is possible to apply defoliants four to five days earlier than farmers' spraying date in field production to avoid weather risks and ensure timely leaf drop without loss of yield.

1. Introduction

Cotton (*Gossypium hirsutum L.*) is a main crop in the textile industry and an important cash crop worldwide. Xinjiang has become a major cotton-growing area and China's largest cotton-producing region (Hu et al., 2021). With the widespread application of machine-harvested cotton in Xinjiang (Tian et al., 2017; Du et al., 2014), the application of chemical defoliants (harvest aids) has gradually become a dominant cultural practice to enhance the quality of machine-harvested cotton due to the limited of mixed green leaves of seed cotton and the bolls that are not fully open (Tian et al., 2020; Du et al., 2013). Xinjiang is located inland in the northwestern region of China, with dry and rainless conditions and a short frost-free period (Li et al., 2020), which limits the efficiency and spraying time of defoliants due to insufficient thermal resource and rapid temperature drop at the time of spraying defoliants, mid to late September (Song et al., 2022). In China, mechanical

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https://doi.org/10.1016/j.fcr.2024.109304
Received 11 September 2023; Received in revised form 5 February 2024; Accepted 8 February 2024
Available online 23 February 2024
0378-4290/

harvesting is recommended at more than 90% defoliation and 95% boll opening percentage (Zhang et al., 2019). Therefore, optimizing the timing of spraying is a great challenge not only to determine the time to reach mechanical harvesting but also to specify the response time of machine-harvested cotton to defoliants under variable environmental conditions.

Defoliating and promotion of cotton boll opening before mechanical harvesting is a crucial agronomic practice to ensure clean fibers and maximize harvest efficiency (Meng et al., 2021; Wang et al., 2023). Several popular defoliants have been tested worldwide. Chemical defoliants, such as thidiazuron and dimethipin, are widely used in cotton production to facilitate machine harvesting, which can promote endogenous ethylene synthesis and inhibit auxin transport, and induce early leaf abscission (Du et al., 2014; Li et al., 2022; Jin et al., 2020). Ethephon as a boll opener can improve boll opening by increasing the concentration of ethylene in the leaves and reducing boll moisture content (Xu et al., 2019; Yu et al., 2022b). Thidiazuron and ethephon are widely mixed as defoliants had been proven to accelerate defoliation and boll opening simultaneously at harvest time. (Meng et al., 2021; Snipes and Baskin, 1994). The effectiveness of defoliants is often limited by environmental factors in northerly regions, such as the rapid drop in temperatures after mid-September and restricted harvest schedules (later September to early October) (Meng et al., 2019). Defoliants are most effective in warm and sunny weather, while cold temperatures generally reduce the efficacy of defoliants (Wright et al., 2015; Song et al., 2022). Temperatures above 18C are considered particularly important during defoliation (Cathey et al., 1985; Shu et al., 2022). Therefore, in order to reduce the risk of adverse weather conditions, it is highly desirable for producers to harvest as early as possible while ensuring that the crop is sufficiently mature.

Proper timing of defoliation balances the rate of increase in yield with the loss of fiber quality (Faircloth et al., 2004b). Premature defoliation can seriously affect crop photosynthesis and transpiration (Quentin et al., 2011), which affects boll formation and results in yield loss in cotton (Bange and Long, 2011; Long and Bange, 2011). Delaying defoliation allows immature boll development, resulting in potentially increased yield (Russell et al., 1993), but fails to ensure timely leaf abscission, resulting in increased seed cotton trash content (Gormus et al., 2017), and increased risk of early frost and adverse weather. There are some methods used by cotton growers to determine when defoliants can be applied to ensure sufficient maturity of cotton at harvest, including the percent of open bolls (POB) (Snipes and Baskin, 1994), nodes above cracked boll (NACB) (Faircloth et al., 2004a, 2004b) and boll cutting technique (Bange and Long, 2011). Previous studies suggest that defoliants could be sprayed to maximize yield when approximately 60% of cotton bolls are open, or four to five nodes above cracked boll (Wright et al., 2015). However, due to the variable weather and unsuitable low temperature in Xinjiang after mid-September, defoliants are often applied before 60% boll opening. However, there is little quantitative information on exactly how far in advance the defoliants can be applied.

Leaf abscission occurs in an abscission zone (AZ), composed of a few layers of small and dense cytoplasm cells, which develops at the junction between the petiole and the stem (Patterson, 2001). Once abscission is triggered, hydrolytic enzymes dissolve the medial layer in the area, resulting in abscission (Olsson and Butenko, 2018). Thidiazuron (TDZ) can increase the concentration of ethylene in the petiole and promote the activation of the abscission zone (Suttle, 1985). Previous studies found that TDZ spraying of cotton leaves promoted the formation of abscission zones (AZ) at 48 hours and leaf abscission at 96 hours (Li et al., 2022), and there was a positive correlation between cotton transpiration and defoliation (Jin et al., 2020). Therefore, transpiration (*Tr*) can be used as an important and reliable indicator for evaluating cotton leaf abscission sensitivity (Jin et al., 2021). In addition, cotton boll development largely depends on the carbon provided by leaf uptake of carbon dioxide for photosynthesis (Zhao et al., 2019), therefore changes in transpiration (*Tr*) at the leaf surface can also be an important factor influencing crop yield. There is a large body of physiological and molecular knowledge on how transpiration responds to defoliants spraying (Jin et al., 2020, 2021), but there is little information on the timing of the response and when 90% defoliation (match machine-harvested cotton) can be achieved, especially under the cultivation conditions and climate in Xinjiang. Accurate measurement of crop transpiration is quite difficult as it is highly influenced by the climate, soil and, agronomic management. The Heat Ratio Method (HRM) has been reported to determine cotton sap velocity and calculate transpiration with high accuracy and sensitivity (Chen et al., 2022).

Therefore, the objective of this study were (a) to quantify the effects of row spacing, plant density, defoliation treatment and their interactions on defoliation percentage, boll open percentage and cotton yield; and (b) to explore how much defoliants spraying could be advanced without yield loss in machine-harvested cotton.

2. Materials and methods

2.1. Experimental site

A field experiment was conducted at Wulanwusu Agro-meteorological Experiment Station (44°17′N, 85°49′E) in Xinjiang, China, in 2020 and 2021. Wulanwusu was located in the south Junggar Basin area in Northwest China and had a temperate continental climate. The soil texture in the 0–40 cm layer at the experimental site was sandy loam with a bulk density of 1.41 g cm^{-3}. The soil had 17.0 g kg^{-1}organic matter, 0.91 g kg^{-1} total *N* content, 84.0 mg kg^{-1} alkali soluble *N*, 91.5 mg kg^{-1} available *P*, and 315 mg kg^{-1} available *K*. Monthly mean air temperature, total precipitation and sunshine hours during the cotton growth period (April to October) in 2020 and 2021 were shown in Table 1. The average air temperature, radiation, relative humidity and wind speed during the defoliation period in both yeas were 18.5°C, 210 W m^{-2}, 47.7% and 1.5 m s^{-1} respectively.

2.2. Experimental design

The field experiment was laid out as a split plot design with three replicates. The main plot treatment was row configuration, which were divided into equal row spacing and narrow-wide row spacing based on the planting requirements for mechanical harvesting. The sub-plot treatments were plant density and defoliation treatment. The equal row spacing (ERS) consisted of 3 rows of cotton with a row spacing of 76 cm; while narrow-wide row spacing (NWRS) consisted of 6 rows with alternating 10 cm narrow and 66 cm wide rows. The total strip width for the two row configuration was 2.28 m. The plant distances in equal row spacing were 7.5 cm and 15 cm, resulting in plant densities of 10 (Low) plant m^{-2} and 18 (High) plant m^{-2}. In narrow-wide row spacing, the plant distances were 6.6 cm and 13.2 cm, which caused plant densities to be 15 (Low) plant m^{-2} and 25 (High) plant m^{-2}. Defoliation treatments

Table 1
Monthly weather data from snowing to harvest in Shihezi, Xinjiang in 2020 and 2021.

Month	Air temperature (°C)		Rainfall (mm)		Sunshine hours (h)	
	2020	2021	2020	2021	2020	2021
April	17.4	13.1	0.4	1.1	270	256
May	22.6	21.3	3.2	28.4	320	317
June	23.4	23.4	29.4	4.3	304	298
July	24.9	27.2	14.1	15.5	299	313
August	23.8	24.1	12.5	15.6	307	326
September	16.9	19.3	1.9	3.2	244	285
October	8.1	7.0	7.7	14.7	244	110
Total*	19.6	19.3	69.2	82.8	1988	1905

* The air temperature is averaged daily, rainfall and sunshine hours are cumulative amounts.

were carried out with an application of 1800 g ha^{-1} Xinsaili (a newly developed harvest aid of 10% thidiazuron and 40% ethephon by Hebei Guoxin Nuonong Biotechnology Co., Ltd.) and water as control. The defoliants were sprayed by a backpack hand sprayers sprayer on 31 Aug 2020 and 6 Sep 2021, respectively. In addition, chemical topping was applied in all plots by an electric knapsack sprayer to restrict the vegetative growth on 10 July 2020 and 11 July 2021.

The total area of each sub-plot was 31.5 m^2 (7 m long and 4.5 m wide). The cotton tested in both years was the same cultivar (Xinluzao 78), and it was sown through holes in plastic film mulch by machine on 24 April in 2020 and 26 April in 2021 respectively and harvested on 10 October 2020 and 20 October 2021. In addition, 420 g ha^{-1} mepiquat chloride was applied six times during the cotton growing season in all plots. Other field management measures were according to farmers' practice.

The experiments were irrigated using a surface drip irrigation system under mechanical cover with plastic film. Irrigation was given as required six to seven times in 2020 and 2021 during crop growing season, with an amount of 480 mm in both years. Fertilizer was applied through drip irrigation system with N (680 kg ha^{-1}), P (270 kg ha^{-1}) and K (80 kg ha^{-1}) during both growing seasons. Fertilizer application and irrigation were based on farmers' practices and crop demand.

2.3. Measurements of leaf area and yield

Three plants were selected from each plot to measure leaf length and width with rulers on 20 August 2020 and 23 August 2021, when leaf area was at its maximum. The leaf area for each plant was calculated as leaf length × width × 0.83 (Zhang et al., 2008).

Cotton lint yield was measured in the blocks with a 2 m×2 m sampling area at the center of each plot, and harvested by hand-picking at harvest times. The seed cotton was sun-dried to a water content of 12%.

2.4. Defoliation efficacy and boll opening percentage

Before spraying, 10 representative cotton plants were randomly selected and marked from two rows at the center of each plot to evaluate the effect of defoliants on leaf defoliation efficacy and boll opening percentage. The number of green leaves, total bolls, and open bolls were marked and recorded before spraying, and the remaining green leaves and open bolls of the marked plants were recorded four times in 2020 (5, 10 15, and 21 days after treatment) and three times in 2021 (7, 14 and 21 days after treatment), respectively. The defoliation percentage was calculated by Eq.1, and the boll opening percentage was calculated by Eq.2.

$$\text{Defoliation percentage}(\%) = \frac{L_a - L_b}{L_a} \times 100\% \tag{1}$$

$$\text{Boll opening percentage}(\%) = \frac{O_b}{O_a} \times 100\% \tag{2}$$

where L_a is the total number of leaves before spraying and L_b is the number of remaining leaves at 21 days after spraying. O_b is the number of open bolls at 21 days after spraying and O_a is the total number of bolls before spraying.

2.5. Sap velocity and stem water potential

Sap velocity was measured by a sap flow meter (SFM1, ICT International, Armidale, Australia) from defoliant application to harvest time in 2020 (August 31 to September 25) and 2021 (September 6 to October 1). For each treatment, one plant in the center row was selected to install the SFM1. The stem diameters of the bottom stem were measured when SFM1 was installed. In 2021, a PSY1 stem water potential sensor (ICT International, Armidale, Australia) was installed on the trunk of the plant near SFM1 to verify whether the plant was under water stress. The data of SFM1 and PSY1 were recorded every 30 min, and the power supply was solved by a solar panel.

The SFM1 used the heat ratio method (HRM) to determine the sap velocity in plants by measuring the ratio of the temperature rise of two temperature sensor probes at the same distance downstream and upstream of the pulse release (Burgess et al., 2001; Madurapperuma et al., 2009).

Daily transpiration per unit ground area (DTr , mm d^{-1}) was calculated by the daily transpiration per plant (Tr , cm^3 d^{-1} $plant^{-1}$) and the plant population density (PPD, plant m^{-2}) (Chen et al., 2022):

$$DTr = V_h \times 3.14 \times (D/2)^2 \times 24 \times PPD \times 10^{-3} \tag{3}$$

where the daily transpiration per plant was produced by the daily average sap velocity (V_h, cm hr^{-1}) and the sap area of each plant within 24 h, D (cm) is the mean stem diameter of three plants measured in each plot used to calculate the sap area.

Daily transpiration per unit leaf area (DTr_{leaf}, cm^3 m^{-2}) was calculated by plant transpiration (Tr , cm^3 d^{-1} $plant^{-1}$) and leaf area per plant in Eq. 4. Since we only have leaf area experiment data when leaf area reached its maximum before spraying, therefore the leaf area per plant was calculated by the number of remaining leaves (N_D, no. plant) and average leaf area per leaf after defoliant application.

$$DTr_{leaf} = \frac{T_r}{(LA/N_L) \times N_D} \tag{4}$$

where LA (m^2 $plant^{-1}$) and N_L (no. plant) are leaf area per plant and the number of leaves per plant on 20 August in 2020 and 23 August in 2021, respectively, which were used to calculate the leaf area per leaf, N_D (no. plant) is the number of green leaves after treatment.

2.6. Data analysis

Exponential regressions were utilized to quantify the relationship between leaf number and the days after spraying defoliants. The equation for the number of leaves was a decreasing negative exponential function (Eq. 5).

$$Y_{leaf} = Y_{leaf\_min} + (Y_{leaf\_max} - Y_{leaf\_min}) \times e^{(-k \times DAS)} \tag{5}$$

where Y_{leaf} represents the number of leaves per unit ground area after defoliants application. DAS is the days after spraying. $Y_{leaf\ max}$ represents the number of leaves per unit ground area before application of defoliants, $Y_{leaf\ min}$ represents the number of leaves per unit ground area after defoliants application. The parameter k (dimensionless) represents the coefficient of defoliation.

To determine the appropriate time for mechanical harvesting, we found the time when the defoliation percentage was about 90% through the fitted curves, i.e., the time when machine harvesting could be started.

2.7. Statistical analysis

Root mean square error ($RMSE$) and normalized root mean square error ($nRMSE$) were used to assess the fit relationship.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(S_i - O_i)^2} \tag{6}$$

$$nRMSE = \frac{RMSE}{O_{mean}} \times 100\% \tag{7}$$

where S_i is the fitted value, O_i is the observed data, n is the number of samples tested, and O_{mean} is the average of all observations.

The cotton leaves changes was fitted by exponential regressions in SPSS 24 (IBM, USA). Treatment effects on defoliation percentage, open bolls percentage, lint yield and fitting parameters (a, b and k) in two years were analyzed by using the General Linear Model (GLM) procedure in SPSS. Defoliation treatment, row spacing, plant density and year were entered as fixed factors, including all interactions, while replicate was entered as a random factor, nested within year (replicate (year)). Defoliation treatment effects on defoliation percentage, open bolls percentage, lint yield and fitting parameters (a, b and k) within each combination of row spacing and plant density and year were analyzed using one-way ANOVA in SPSS. Origin 2021 (OriginLab Corporation, Northampton, MA, USA) was used for graphing.

3. Result

3.1. Yield

Lint cotton yield was significantly affected by defoliants compared to control, which was 14.8% (2475 kg ha^{-1}) higher than in control (2156 kg ha^{-1}), but did not interact with year (Tables 2 and 3. 4). The lint cotton yield was not affected ($P > 0.05$) by row spacing but was affected by the interaction with year (Table 2). Plant density and its interaction with year had no effect ($P > 0.05$) on lint yield. However, the lint yield differed between the two years and was higher in 2021 (2593 kg ha^{-1}) than in 2020 (2038 kg ha^{-1}) due to differences in cotton climate and management (Tables 2 and 3).

3.2. Defoliation

During the harvest time, the mean defoliation percentage in all defoliant treatments was 90.6%, which met the requirements for mechanical harvesting and was much higher ($P < 0.01$) than in the control (60.1%) average across the two years (Tables 2 and 3). Defoliation percentage was significantly affected by the interaction between row spacing and plant density (Table 2).

The number of remaining total leaves per unit ground area in both years fitted well (nRMSE < 12% and $R^2 > 0.94$) a declining negative exponential relationship with the days after spraying (Fig. 1). Before spraying, the total number of leaves per unit ground area with defoliation treatment was comparable with the control, but was significantly affected by the interaction of row spacing and plant density. Leaf dropping was significantly accelerated by the spraying of defoliant, resulting in a significantly different *k* value in the fitted equation (Tables 2 and 3).

3.3. Boll opening

As shown in Fig. 2, the number of open bolls increased in all treatments compared to the control after application of defoliants. Defoliant application also accelerated the speed of boll opening, especially from 0 to 10 days in 2020 and 0–14 days in 2021 after application (Fig. 2). At 21 days after spraying, the boll opening percentage across two years was 9.83% higher in defoliant treatment than in control, which did not depend on the year (Tables 2 and 3). There were no significant effects of row spacing and plant density and their interaction on boll opening percentage.

3.4. Sap velocity and stem water potential

Sap velocity was recorded half hourly during the defoliation period and showed significant temporal variability in each treatment (Fig. 3a, b, g, and h). The sap velocity in defoliant treatment (on average 3.99 cm hr^{-1}) was lower than in control (8.37 cm hr^{-1}) (Fig. 3). The sap velocity was also significantly affected by plant density, under the narrow-wide row spacing in 2020, the mean sap velocity at a density of 15 plant m^{-2} with a value of 6.16 cm hr^{-1}, started to decrease at 5 days (Fig. 3a and g) after defoliant application, while at a density of 25 plant m^{-2} with a value of 5.01 cm hr^{-1}, decreased at 4 days (Fig. 3b and h) due to its smaller LAI.

An opposite trend to sap velocity was observed for stem water potential in cotton. At the first four days in equal row spacing (18 plant m^{-2}) in 2021 after applying, there was no variation in sap velocity, with water potential approaching zero at night and reaching −7 to −6 MPa at noon. However, after 4 days of defoliant treatment, stem water potential reached a peak value of −2 to −2.5 MPa at noon with the decreased sap velocity and then gradually tended to 0 MPa (Fig. 4).

3.5. Daily transpiration per unit ground area and unit leaf area

The daily transpiration per unit ground area (*DTr*) during the defoliation period differed significantly between defoliant treatment and control (Fig. 5). On the first four to five days, the *DTr* in defoliant treatment was higher than in control and then decreased rapidly. The mean *DTr* in defoliant application on average was 1.26 mm d^{-1}, while in control was 2.07 mm d^{-1} over the two years. The mean *DTr* during the defoliation period in 2021 (1.88 mm d^{-1}) was higher than that in 2020 (1.40 mm d^{-1}).

The DTr_{leaf} in defoliant treatment (0.81 mm d^{-1}) was 11% lower than in control (0.91 mm d^{-1}), but was sometimes much higher, for example, between 20 and 22 DAS in 2020, which may be due to higher temperatures (above 18 °C) (Fig. 6a). The DTr_{leaf} was not significantly different between the two years, average 0.85 mm d^{-1} in 2020 and 0.87 mm d^{-1} in 2021 (Fig. 6).

Table 2
The *P* values of ANOVA for the effects of row configuration, defoliation treatment, plant density, year and their interactions on defoliation percentage, open bolls percentage, lint yield, and the fitted parameters of exponential regressions at Wulanwusu, Xinjiang, in 2020 and 2021.

Effect	Defoliation percentage	Open bolls percentage	Lint yield	Leaves m^{-2}		
				Y_{leaf_max}	Y_{leaf_min}	k
Year	ns	ns	*	*	ns	ns
Replicate (Y)	ns	ns	ns	ns	ns	ns
Row spacing×Replicate (Y)	ns	ns	ns	ns	ns	ns
Row spacing	ns	ns	ns	ns	*	*
Defoliation	**	**	*	ns	**	**
Plant density	ns	ns	ns	**	**	ns
Year×Row spacing	ns	ns	**	ns	ns	**
Year×Defoliation	ns	ns	ns	**	ns	ns
Year×Plant density	ns	ns	ns	ns	ns	ns
Row spacing×Defoliation	ns	ns	ns	**	ns	**
Defoliation×Plant density	ns	ns	ns	*	**	**
Plant density×Row spacing	**	ns	ns	ns	ns	*
Row spacing×Defoliation×Plant density	ns	ns	ns	ns	ns	ns

Analyses were made using a split block design with row spacing as main plot factor and defoliation treatment and plant density as subplot factors.
ns indicates $p > 0.05$, * indicates $p < 0.05$, and ** indicates $p < 0.01$.

Table 3
Yield, defoliation and boll opening percentage in relation to row configuration, defoliation method, and plant density treatments in Shihezi, Xinjiang in 2020 and 2021.

Year	Row spacing	Plant density	Defoliation	Defoliation percentage (%)	Open bolls percentage (%)	Lint yield (Kg ha^{-1})
2020	Equal row spacing	Low	Defoliants	88.9a	84.8a	2283a
			Control	52.8b	72.2a	1717b
			SE	3.10	5.95	124
	Equal row spacing	High	Defoliants	96.8a	82.4a	1983a
			Control	58.0b	74.6a	1767a
			SE	2.37	3.98	189
	Narrow-wide spacing	Low	Defoliants	90.8a	85.1a	2300a
			Control	67.1a	79.5a	2150a
			SE	6.67	1.75	195
	Narrow-wide spacing	High	Defoliants	84.1a	84.1a	2133a
			Control	65.1b	77.4b	1967a
			SE	1.76	1.37	176
2021	Equal row spacing	Low	Defoliants	89.0a	84.6a	3017a
			Control	57.0b	76.1a	2627b
			SE	3.95	3.08	92.8
	Equal row spacing	High	Defoliants	94.4a	87.4a	2950a
			Control	62.3b	83.4a	2717a
			SE	2.00	4.87	160
	Narrow-wide spacing	Low	Defoliants	94.1a	88.1a	2800a
			Control	66.1b	80.1a	2317a
			SE	3.53	4.78	227
	Narrow-wide spacing	High	Defoliants	86.4a	82.9a	2333a
			Control	52.6b	75.4b	1983b
			SE	4.86	1.36	80.8

Same small letters indicate no significant difference between defoliation treatment within the same row configurations and plant densities at a=0.05 level.

Table 4
Fitted parameters of exponential regressions for the number of leaves in cotton under different row configuration, defoliation method, and plant density treatments in Shihezi, Xinjiang, China in 2020 and 2021.

Year	Row spacing	Plant density	Defoliation	Leaves m^{-2}			
				Y_{leaf_max}	Y_{leaf_min}	k	nRMSE
2020	Equal row spacing	Low	Defoliants	338a	29.6b	0.19a	3.78
			Control	375a	89.8a	0.06b	6.04
			SE	14.3	11.5	0.02	
	Equal row spacing	High	Defoliants	727a	37.6b	0.22a	7.37
			Control	703a	234a	0.10b	4.77
			SE	17.9	45.2	0.02	
	Narrow wide row spacing	Low	Defoliants	737a	23.8b	0.14a	4.92
			Control	645b	118a	0.07b	2.38
			SE	7.90	23.1	0.01	
	Narrow wide row spacing	High	Defoliants	1101a	64.7b	0.09a	18.2
			Control	912a	289a	0.11a	6.79
			SE	55.8	45.1	0.01	
2021	Equal row spacing	Low	Defoliants	275a	12.2a	0.14a	2.27
			Control	372a	94.7a	0.07a	2.12
			SE	30.0	23.2	0.02	
	Equal row spacing	High	Defoliants	659a	6.99b	0.15a	4.49
			Control	613a	217a	0.11b	2.27
			SE	19.2	19.0	0.01	
	Narrow wide row spacing	Low	Defoliants	621a	36.1b	0.15a	5.62
			Control	537a	141a	0.11b	3.47
			SE	61.1	16.9	0.01	
	Narrow wide row spacing	High	Defoliants	941a	9.14b	0.10a	11.1
			Control	816a	283a	0.12a	1.80
			SE	46.8	31.9	0.02	

Same small letters indicate no significant difference between defoliation treatment within same row configurations and plant densities at a=0.05 level.

4. Discussion

Spraying defoliants increased cotton yield by 14.8%, defoliation percentage by 50.4%, boll opening percentage by 9.8% and accelerated leaf shedding and boll opening compared to the control at harvest time, which was more beneficial for machine-harvested cotton. The sap velocity was lower in defoliation treatment than in the control. The daily transpiration per unit ground area (*DTr*) and leaf area (DTr_{leaf}) over two years was lower in defoliant treatment than in control. The *DTr* was not affected by row spacing, but the DTr_{leaf} was significantly lower in equal row spacing than in narrow-wide rows. The *DTr* did not change significantly in the first 4–5 days after spraying defoliants, and then gradually decreased. We recommend that the timing of spraying defoliants be properly advanced based on transpiration sensitivity to defoliants, field weather conditions, and empirical harvest times.

An appropriate defoliation strategy plays a crucial role in determining yield, fiber quality, and net returns. Farmers usually apply defoliants when open bolls reach 60% to ensure cotton is sufficiently maturity without yield loss (Bynum and Cothren, 2008). However, our study differs from previous findings, as we observed no yield loss even

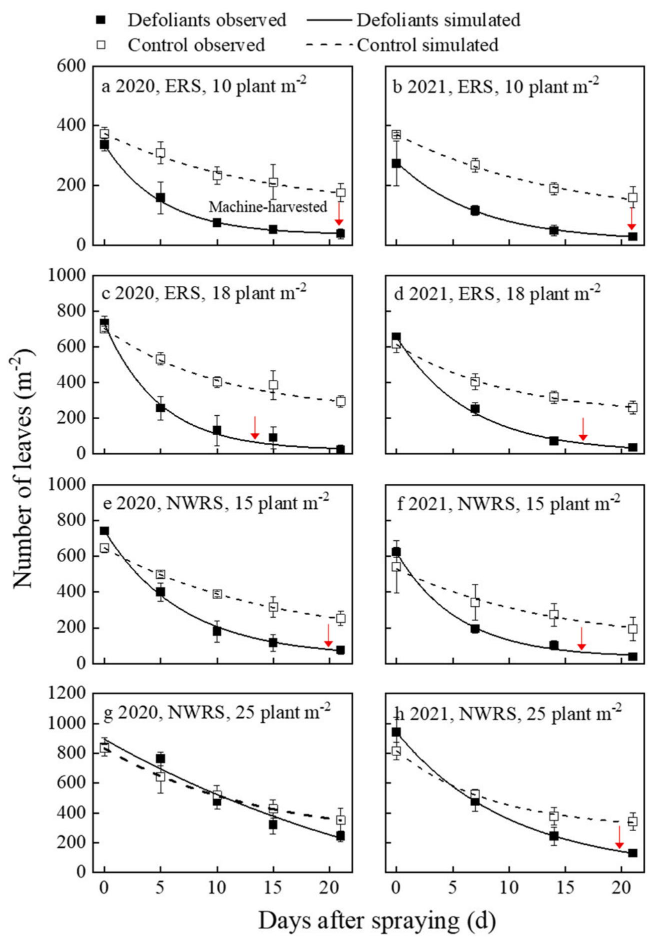

Fig. 1. Fitted (lines) and observed (points) leaves per unit ground area in cotton under different defoliation methods, row configurations (ERS: equal row spacing, NWRS: narrow-wide row spacing) and plant densities in 2020 and 2021. The red arrow indicated the time when the defoliation percentage reaches 90%.

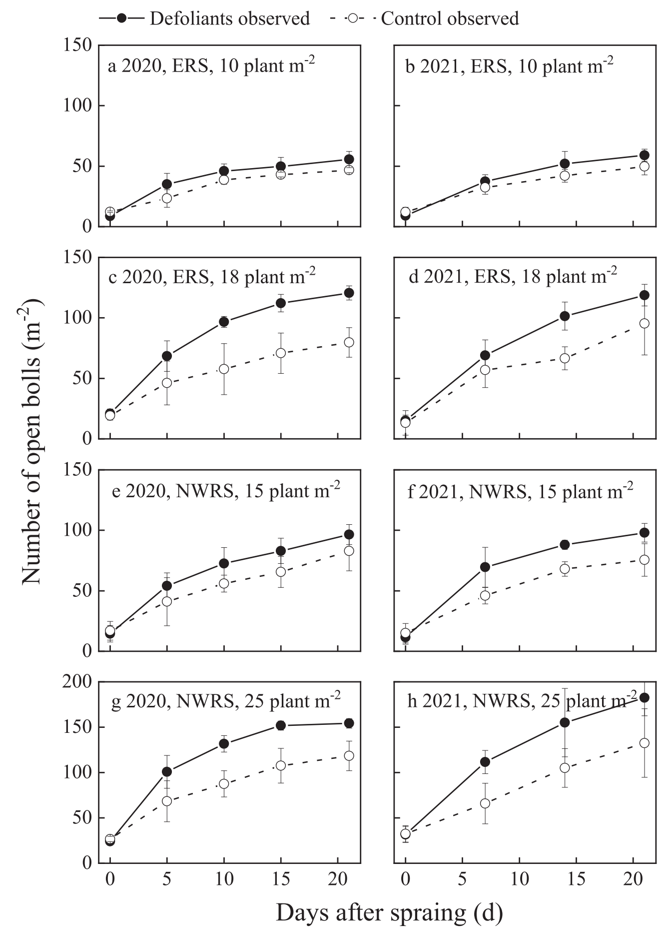

Fig. 2. The number of open bolls per unit ground area in cotton under different defoliation methods, row configurations (ERS: equal row spacing, NWRS: narrow-wide row spacing) and plant densities in 2020 and 2021.

though defoliants were applied at approximately 18% of open bolls in both years (Table 3; Fig. 2). This could be attributed to the suitable temperature during defoliant period in both years (Fig. 3), also by the dosage and variety of defoliant. The 14.8% increase in yield after defoliant spraying may be due to the reduction in plant leaf area, which improves ventilation and light penetration and reduces the incidence of fungal diseases after defoliation. Additionally, defoliants contain ethephon, which promoted boll opening by increasing the production of ethylene inside the bolls, thus increasing yields (Rajni and Brar, 2011). Differences in cotton yield between the two years may be due to various environmental and agronomic factors.

The requirements for machine-harvested cotton, the defoliation percentage and boll opening percentage must be at least 90% (Meng et al., 2019; Yu et al., 2022a). Spraying a mixture of thidiazuron and ethephon on cotton resulted in rapid green leaves drop and improved machine harvesting efficiency (Xu et al., 2019). This was consistent with our results that the application of defoliant accelerated the defoliation process (Fig. 1), allowing an earlier cotton harvest to avoid early frosts and inclement weather that often occur in Xinjiang, e.g., the defoliation percentage can reach 90% on the 14th day after spraying at equal row spacing with 18 plant m^{-2} in 2020 (Fig. 1). Even though the sample size used for the fitting for 2021 was only four, the data points showed a clear trend, which led to a fitted curve useful to derive the moment of 90% defoliation. However, the mean boll opening percentage after 21 days of defoliation treatment was less than 90% (Table 3, Fig. 2), which still needs to be improved. The application of chemical topping at the flowering stage could also explain the high rate of leaf drop and boll opening, as the canopy width is significantly reduced, promoting compactness of the plant type and exposing more leaves and bolls in the middle and lower canopy layers, increasing the efficiency of defoliants. (Yu et al., 2022a; Ren et al., 2013; Dai et al., 2022). Weather conditions, specifically temperature and rainfall, have a significant impact on defoliation efficiency during the defoliation period (Snipes and Cathey, 1992; Cathey, 1985). 18 °C is the minimum temperature required for leaves to respond to most defoliants (Shu et al., 2022). In our experiments, the average daily temperature during defoliation was above 18 °C in both 2020 and 2021, which can explain the lack of significant differences in defoliation and boll opening percentage between the two years.

Defoliants increase the synthesis of ethylene and interrupt of auxin flux, activating the release layer where petioles attach to branches or main stems, leading to the formation of an abscission zone and leaf fall (Morgan et al., 1977; Xu et al., 2019). Paraffin sections showed that the formation of abscission zones (AZ) on cotton petioles after TDZ treatment occurred at 3–5 days (Xu et al., 2019). In our study, the transpiration began to decrease at 4–5 days after spraying the defoliant, which indicated that the AZ had formed and the leaves began to drop at this time (Fig. 3; Fig. 5). During the formation of the AZ period, cotton transpiration was unaffected (Fig. 5), indicating that the vascular bundle remains intact and delivers water and nutrients to the developing boll, and boll opening increased from 18.1% to 62.7%, ensuring no loss of yield (Fig. 2). Therefore, based on experience harvest time, we recommend spraying defoliant 4–5 days in advance. In addition, the cotton abscission zone formation and the progression of defoliation also depend

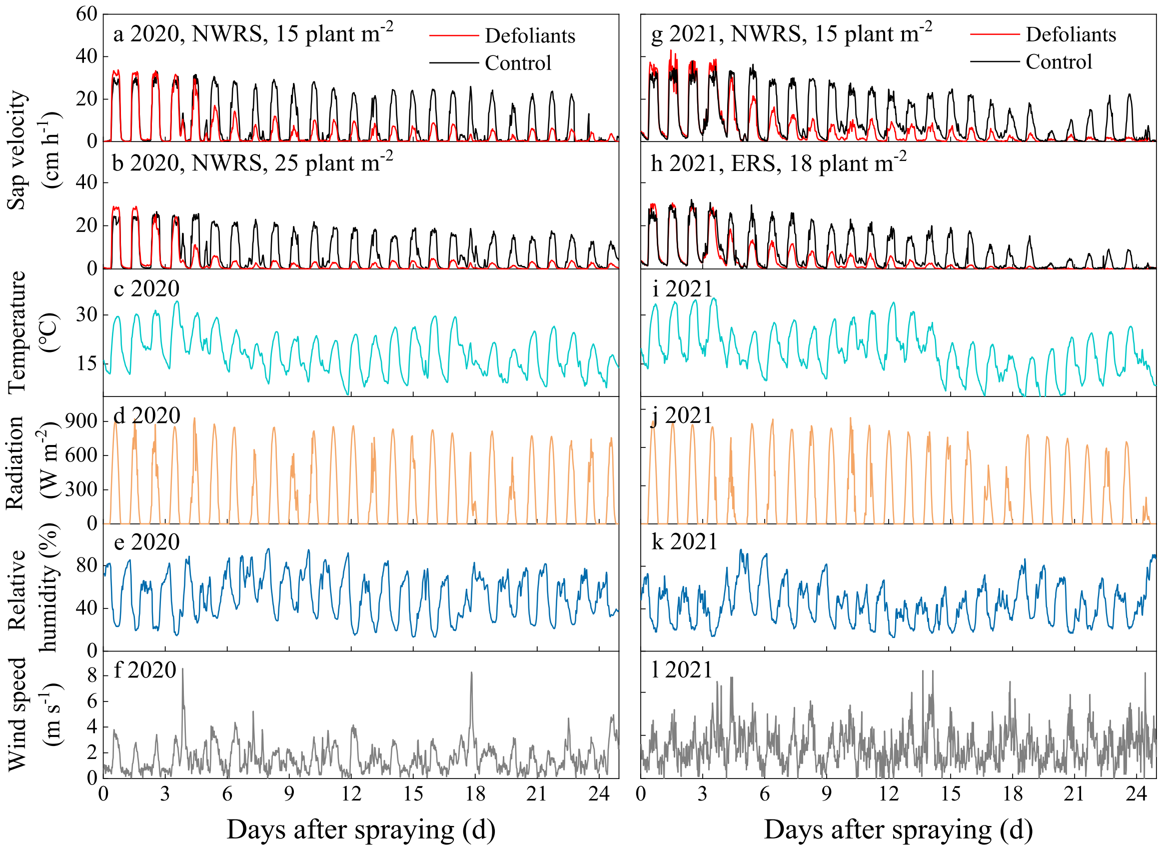

Fig. 3. Sap velocity and environmental conditions during the defoliation period for defoliation, row spacing (ERS: equal row spacing, NWRS: narrow-wide row spacing) and plant density treatments in Shihezi, China in 2020 and 2021.

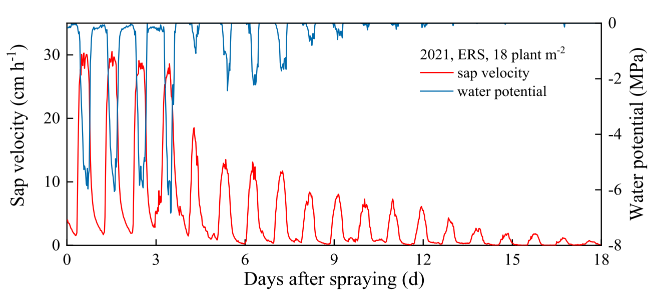

Fig. 4. Diurnal course of stem sap flow and water potential during cotton defoliation stage in equal row spacing with a plant density of 18 plants m^{-2} in 2021. The sap flow data are the same as in Fig. 3.

on leaf abscission sensitivity, which varies with variety and dosage (Jin et al., 2021). Previous studies have shown that an increase in ethylene content in cotton petioles promotes leaf abscission in water-stressed plants (Morgan et al., 1977). We found that the stem water potential reached a maximum of −7 MPa at midday before spraying, when cotton was in a water-deficit stress, it changed from −7 MPa (water deficit) to −2.5 MPa (normal) when transpiration began to decline 4–5 days after spraying (Fig. 4).

Spraying defoliants will not only affect the defoliation, but also affect the physiological functioning of leaves, leading to reduced transpiration. According to Jin et al. (2020), their study on dissected cotton leaves revealed that TDZ-treated leaves experienced damaged the cells and stomata, inhibiting the channels through which plants exchange photosynthetic CO_2 gases and control transpiration, which also led to the leaves eventual loss water and fall off. These findings align with our findings, which showed that daily transpiration per unit leaf area was 11% lower in the defoliation treatment than in the control (Fig. 6). Temperature can also influence transpiration in crops by directly affecting stomatal function (Kostaki et al., 2020). Since the climatic conditions during defoliation were similar in both years, the impact of temperature on crop transpiration was relatively diminished. However, the available information is limited and this part requires further research.

5. Conclusions

Applying of defoliants promoted defoliation and boll opening without yield loss. Changes in cotton transpiration were measured using the heat ratio method and it was found that the application of harvest aids had a small effect on transpiration within 4–5 days. Thus, we can combine the local climate conditions and properly spray the defoliants 4–5 days earlier than farmers' spraying date in the production as appropriate to ensure that the green leaves fall off before mechanical harvesting and will not affect the cotton yield and quality. The results of this study would guide for determining the appropriate spraying time that is conducive to mechanical harvesting.

CRediT authorship contribution statement

Ji Chunrong: Investigation. **Chen Yongfan:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Zhang Lizhen:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Evers Jochem B.:** Writing – review & editing. **Xiang Dao:** Project administration. **Li Jie:** Investigation. **Yang Mingfeng:** Validation, Project administration, Investigation. **Wang Xuejiao:** Project administration, Investigation. **Zhang Zeshan:** Investigation. **Wang Sen:** Project administration, Investigation. **Ji Fen:** Project administration, Investigation. **Sun Shuai:**

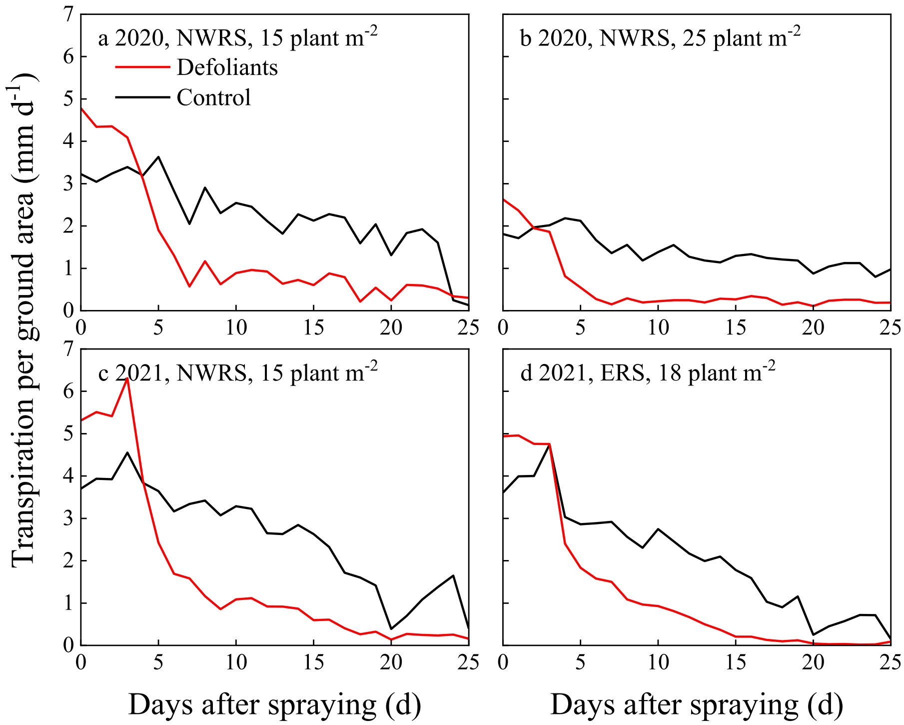

Fig. 5. Daily transpiration per unit ground area during defoliation stage in cotton under different defoliation methods, row configurations (ERS: equal row spacing, NWRS: narrow-wide row spacing) and plant densities in 2020 and 2021.

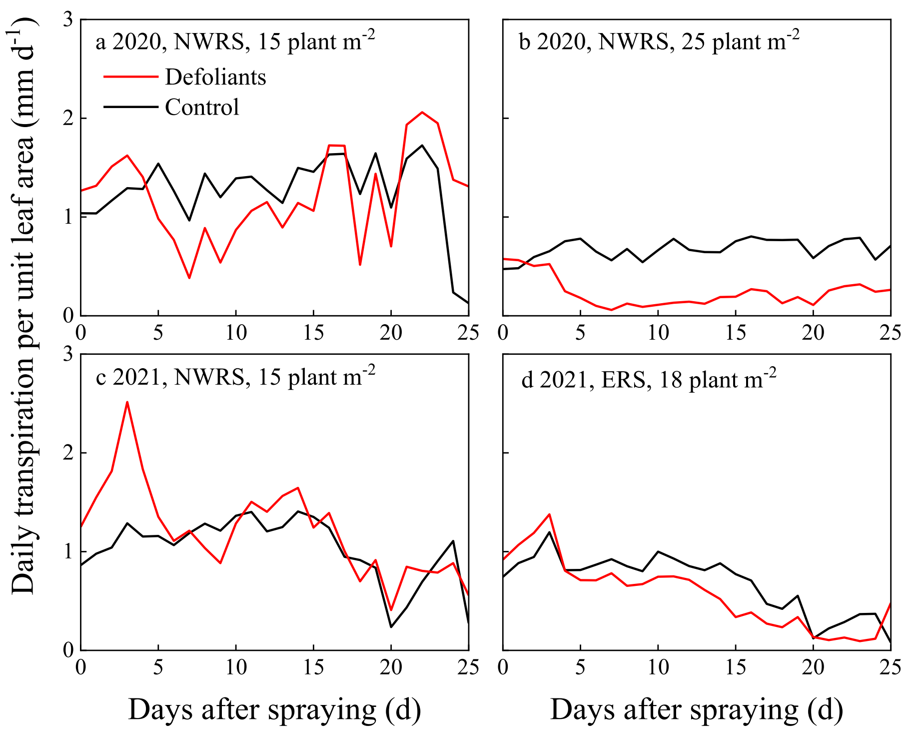

Fig. 6. Daily transpiration per unit leaf area during defoliation stage in cotton under different defoliation methods, row configurations (ERS: equal row spacing, NWRS: narrow-wide row spacing) and plant densities in 2020 and 2021.

Investigation. **Zhang Yutong:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

This research was supported by the Xinjiang Science and Technology Major Project (2023A02003-5), the National Natural Science Foundation of China (42105172). Chen Y. obtained a scholarship from China Scholarship Council (CSC202206350092).

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